U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-262 912

TOXICITY OF FOUR PESTICIDES TO WATER FLEAS AND FATHEAD MINNOWS. ACUTE AND CHRONIC TOXICITY OF ACROLEIN, HEPTACHLOR, ENDOSULFAN, AND TRIFLURALIN TO THE WATER FLEA (DAPHNIA MAGNA) AND THE FATHEAD MINNOW (PIMEPHALES PROMELAS)

EG AND G, BIONOMICS, WAREHAM, MASSACHUSETTS

PREPARED FOR
ENVIRONMENTAL RESEARCH LABORATORY
DULUTH, MINNESOTA

NOVEMBER 1976

PB 262 912

EPA-600/3-76-088
November 1976

Ecological Research Series

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-76-099		

4. TITLE AND SUBTITLE	5. REPORT DATE (Issuing date)
TOXICITY OF FOUR PESTICIDES TO WATER FLEAS AND FATHEAD MINNOWS*	November 1976
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Kenneth J. Macek, Mark A. Lindberg, Scott Sauter, Kenneth S. Buxton, and Patricia A. Costa	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
EG & G Bionomics 790 Main Street Wareham, Massachusetts 02571	1BA608
	11. CONTRACT/GRANT NO. Contract 68-01-0738

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Duluth, Minnesota 55804	Final
	14. SPONSORING AGENCY CODE EPA-ORD

15. SUPPLEMENTARY NOTES *Subtitle--Acute and Chronic Toxicity of Acrolein, Heptachlor, Endosulfan, and Trifluralin to the Water Flea (Daphnia magna) and the Fathead Minnow (Pimephales promelas)

16. ABSTRACT

Maximum acceptable toxicant concentrations (MATC's) for each pesticide for both species were estimated by measuring survival, growth and reproduction success as indicators of toxic effects. The MATC for acrolein was estimated to be >16.9 and <33.6 µg/l for daphnids and >11.4 and < 41.7 for fathead minnows, those estimated for heptachlor were >12.5 and <25.0 µg/l for daphnids and >0.86 and <1.84 µg/l for fathead minnows, those estimated for endosulfan were >2.7 and <7.0 µg/l for daphnids and >0.2 and <0.4 µg/l for fathead minnows, and those estimated for trifluralin were >2.4 and <7.2 µg/l for daphnids and >1.9 and <5.1 µg/l for fathead minnows.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pesticides* Toxicity* Bioassay* Acroleins Minnows Daphnia Heptachlor	Acute toxicity Chronic toxicity Endosulfan Trifluralin Aquatic organisms	6F 7C

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	Unclassified	68
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	4.50-3.00

EPA Form 2220-1 (9-73)

☆ U.S. GOVERNMENT PRINTING OFFICE 1976—757-056/5435

EPA-600/3-76-099
November 1976

TOXICITY OF FOUR PESTICIDES TO WATER FLEAS AND FATHEAD MINNOWS

Acute and Chronic Toxicity of Acrolein, Heptachlor, Endosulfan, and Trifluralin to the Water Flea (*Daphnia magna*) and the Fathead Minnow (*Pimephales promelas*)

by

Kenneth J. Macek, Mark A. Lindberg, Scott Sauter,
Kenneth S. Buxton, Patricia A. Costa
E G & G, Bionomics
Wareham, Massachusetts 02571

Contract 68-01-0738

Project Officer

A. Jarvinen
Environmental Research Laboratory-Duluth
Duluth, Minnesota 55804

ENVIRONMENTAL RESEARCH LABORATORY-DULUTH
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MINNESOTA 55804

DISCLAIMER

This report has been reviewed by the Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry---physically and chemically alter lakes, rivers, and stream. Such alterations threaten terrestrial organisms,as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota develops methods, conducts laboratory and field studies, and extrapolates research findings

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man

This report determines the effect of four pesticides on two species of aquatic organisms.

Donald I. Mount, Ph.D.
Director
Environmental Research Laboratory
Duluth, Minnesota

ABSTRACT

Water fleas (Daphnia magna) and fathead minnows (Pimephales promelas) were chronically exposed to various concentrations of acrolein, heptachlor, endosulfan and trifluralin in separate flowing water systems. Exposures were through two complete life cycles for Daphnia and through at least one complete life cycle for fathead minnows.

Maximum acceptable toxicant concentrations (MATC's) for each pesticide for both species were estimated by measuring survival, growth and reproduction success as indicators of toxic effects. The MATC for acrolein was estimated to be >16.9 and <33.6 µg/l for daphnids and >11.4 and <41.7 for fathead minnows, those estimated for heptachlor were >12.5 and <25.0 µg/l for daphnids and >0.86 and <1.84 µg/l for fathead minnows, those estimated for endosulfan were >2.7 and <7.0 µg/l for daphnids and >0.2 and <0.4 µg/l for fathead minnows, and those estimated for trifluralin were >2.4 and <7.2 µg/l for daphnids and >1.9 and <5.1 µg/l for fathead minnows.

This report was submitted in fulfillment of Contract No. 68-01-0738 by E G & G, Bionomics, under the sponsorship of the Environmental Protection Agency. Work was completed in December, 1974.

CONTENTS

SECTION		PAGE
I	Introduction	1
II	Conclusions	5
III	Recommendations	6
IV	Materials and Methods	8
	Exposure Systems	8
	Acute Toxicity Procedures	10
	Chemical Methodology	11
	Statistics	21
	Chronic Exposure	21
	Daphnia magna	21
	Pimephales promelas	22
V	Results	24
	Acute Bioassays	24
	Water Chemistry	24
	Chronic Exposure	29
	Daphnia magna to acrolein	29
	Daphnia magna to heptachlor	29
	Daphnia magna to endosulfan	32
	Daphnia magna to trifluralin	33
	Pimephales promelas to acrolein	35
	Pimephales promelas to heptachlor	38
	Pimephales promelas to endosulfan	38
	Pimephales promelas to trifluralin	42
	Residue Analysis	45
	Calculation of Application Factors	48
VI	Discussion	50
VII	References	53

TABLES

No.		PAGE
1	Chemical analysis of the diluent water utilized during chronic exposures of Daphnia magna and fathead minnows to acrolein, heptachlor, endosulfan and trifluralin.	9
2	Recovery of heptachlor and heptachlor epoxide from quality control water samples.	15
3	Recovery of heptachlor and heptachlor epoxide from quality control tissue samples.	17
4	Recovery of endosulfan from quality control water samples.	19
5	Recovery of trifluralin from quality control water samples.	20
6	Recovery of trifluralin from quality control tissue samples.	21
7	Mean and range of measured concentrations of hardness, alkalinity, acidity, dissolved oxygen and pH from water samples taken periodically during chronic exposure of Daphnia magna to four pesticides.	25
8	Mean and range of measured concentrations of hardness, alkalinity, acidity, dissolved oxygen and pH from water samples taken periodically during chronic exposure of fathead minnows (Pimephales promelas) to four pesticides.	26
9	Nominal and mean measured concentrations of acrolein, endosulfan and trifluralin in water during continuous exposure of Daphnia magna.	27
10	Nominal and mean measured concentrations of acrolein, heptachlor, endosulfan and trifluralin during chronic exposure of fathead minnows (Pimephales promelas).	28
11	Mean percent survival of Daphnia magna continuously exposed to acrolein for 64 days.	30
12	Mean production of young per female Daphnia magna continuously exposed to acrolein for 64 days.	30
13	Mean percent survival of Daphnia magna continuously exposed to heptachlor for 64 days.	31

TABLES

No.		PAGE
14	Mean production of young per female Daphnia magna continuously exposed to heptachlor for 64 days.	31
15	Mean percent survival of Daphnia magna continuously exposed to endosulfan for 64 days.	32
16	Mean production of young per female Daphnia magna continuously exposed to endosulfan for 64 days.	33
17	Mean percent survival of Daphnia magna continuously exposed to trifluralin for 64 days.	34
18	Mean production of young per female Daphnia magna continuously exposed to trifluralin for 64 days.	34
19	Survival and growth of fathead minnows (Pimephales promelas) after 30 and 60 days, and 35 weeks continuous exposure to acrolein.	36
20	Sexual development, spawning, hatchability of eggs, and survival and growth of offspring after 30 and 60 days, for fathead minnows (Pimephales promelas) continuously exposed to acrolein.	37
21	Survival and growth of fathead minnows (Pimephales promelas) after 30 days, 60 days and 40 weeks continuous exposure to heptachlor.	39
22	Sexual development, spawning, hatchability of eggs, and survival and growth of offspring after 30 and 60 days, for fathead minnows (Pimephales promelas) continuously exposed to heptachlor.	40
23	Survival and growth of fathead minnows (Pimephales promelas) after 30 days, 60 days and 40 weeks continuous exposure to endosulfan.	41
24	Sexual development, spawning, hatchability of eggs, and survival and growth of offspring after 30 and 60 days, for fathead minnows (Pimephales promelas) continuously exposed to endosulfan.	43
25	Survival and growth of fathead minnows (Pimephales promelas) after 30 days, 60 days and 61 weeks continuous exposure to trifluralin.	44

TABLES

No.		PAGE
26	Spawning results, egg hatchability, and survival and growth of fathead minnow (Pimephales promelas) fry after 30 and 60 days exposure to various concentrations of trifluralin.	46
27	Mean measured concentrations of heptachlor (+ epoxide) and trifluralin in water (µg/l) and in the eviscerated carcass (µg/kg) of fathead minnows (Pimephales promelas) continuously exposed to the pesticides in separate systems.	47
28	Acute and chronic toxicity values (µg/l) for the water flea (Daphnia magna) and calculated application factors for each pesticide.	48
29	Acute and chronic toxicity values (µg/l) for the fathead minnow (Pimephales promelas) and calculated application factors for each pesticide.	49

ACKNOWLEDGEMENTS

During the period of performance of this research effort we were fortunate to have laboratory assistance from Steve Ells and Emily Dionne who maintained exposure systems and were significantly involved with various aspects of fish chronics, from Gerry LeBlanc who provided expertise in performing the invertebrate chronics, and from Ronni Krasny and Joanna Enos who performed chemical analyses, and to Dr. Sam R. Petrocelli for contructively reviewing the final report.

Finally, our sincere appreciation is extended to Mr. Alfred Jarvinen, Project Officer, Environmental Protection Agency, National Water Quality Laboratory, Duluth, Minnesota for his guidance, patience, and contractual advice during the performance of these studies.

SECTION I

INTRODUCTION

The current concern regarding the protection of aquatic life in surface waters has prompted evaluation of the effects of chemicals on aquatic invertebrates and fishes. Much of the toxicological research with aquatic biota has been limited to the development of acute toxicity values as a measure of the biological effect of chemicals. More recently, utilization of the chronic exposure of fishes and aquatic invertebrates to chemicals has received particular attention due to the numerous parameters that can be evaluated as indices of toxic effects (Mount, 1968; Eaton, 1970; Arthur, 1970; McKim and Benoit, 1971; Arthur et al,. 1973; Macek et al., 1976a, 1976b). The "Laboratory Fish Production Index (LFPI)" as defined by Mount and Stephan (1967) reflects toxic effects on reproduction, growth, spawning behavior, egg mortality, and fry survival. The highest observed toxicant concentration that has no effect on these parameters during continuous chronic exposure is termed the maximum acceptable toxicant concentration (MATC).

Based on available information on occurrence and persistence in the environment, relative toxicity to fishes and aquatic invertebrates, current uses and use patterns, and/or total amount of chemical applied annually, four pesticides were selected for intensive investigation to evaluate the potential chronic effects of these chemicals on aquatic organisms.

Acrolein (acrylic aldehyde) is a herbicide used in bodies of water such as lakes, rivers, canals and irrigation ditches for controlling submerged vegetation and floating plants (Overbeck, 1962). As an example of acrolein's volatility, Battelle (1970) reported that a concentration of 0.7 mg/l of this herbicide in the water of treated irrigation ditches flowing at a rate of approximately 130 cubic feet per second decreased by 98% over a 19 mile stretch at a temperature of 64°F. At a lower and less typical temperature of 48°F, the loss was only 62% over a distance of 27 miles. Burdick et al. (1964) investigated the toxicity of acrolein to brown trout and bluegills by a procedure approximating the concentration to be expected from a single field application under certain conditions. They reported the 24-hour LC50 of acrolein for trout and bluegills to be 46 and 75 µg/l, respectively. They also observed that the threshold for mortality of both species was below the concentration recommended for controlling aquatic vegetation. Louder and McCoy (1962) reported that 24-hour LC50 values for acrolein and largemouth bass, bluegills, bowfin, mosquito fish and fathead minnows ranged from 62-183 µg/l. Little additional information on the effects of acrolein on aquatic organisms is available.

Heptachlor (1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-endo-methanoindene) is an organochlorine insecticide which has been reported to be widely distributed in both terrestial and aquatic environments. The occurrence, translocation, persistence and degradation of heptachlor in soils and crops have been adequately documented (Bruce and Decker, 1966; King et al., 1966; Lichtenstein and Schulz, 1965; and Lichtenstein et al., 1970). Harris and Sans (1972) have described the behavior of the primary metabolite, heptachlor epoxide, in soil. Lichtenberg et al. (1969) have reported on the occurrence of heptachlor and its epoxide in surface waters in the United States; Eichelberger and Lichtenberg (1971) have described the persistence of these compounds in river water, observing that no measurable degradation or chemical change was evident for heptachlor epoxide in raw river water during an eight week period. Henderson et al. (1969) summarized the results of a national monitoring program which consisted of the analysis of 590 composite fish samples collected from 50 stations over a 2-year period. They reported finding heptachlor and heptachlor epoxide at concentrations ranging from 0.01-8.4 mg/kg in 32% of the samples. The results of this national monitoring program were updated (Henderson et al., 1971) and similar observations were reported. Hannon et al. (1970) investigated the distribution of pesticides in water, bottom sediments, zooplankton, algae, crayfish, aquatic insects and fishes. They reported that measurable concentrations of heptachlor and heptachlor epoxide were present in the majority of sample types.

Much research has been conducted on the acute toxicity of heptachlor to aquatic organisms and the effect of water quality on acute toxicity. Sanders and Cope (1966) determined a 48-hour EC50 (95% confidence limits) of 42 (21-63) µg/l for Daphnia pulex exposed to heptachlor. Frear and Boyd (1967) corroborated this estimate of toxicity of heptachlor to daphnids by reporting a 48-hour LC50 of 52 ug/l for Daphnia magna exposed to heptachlor and 120 µg/l for the same species exposed to heptachlor epoxide. Sanders and Cope(1968) reported the 96-hour LC50 of heptachlor for stonefly naiads (Pteronarcys) was 1.1(0.8-1.4) µg/l. The acute toxicity of heptachlor has been reported for freshwater fishes (Henderson et al., 1959) and estuarine fishes (Eisler, 1970), with 96-hour LC50 values ranging from 26-320 µg/l for freshwater fishes. Bridges (1965) investigated the effects of time and temperature on the toxicity of heptachlor to redear sunfish and Eisler (1970) evaluated the effects of temperature, salinity, and pH on the toxicity of heptachlor to the estuarine killifish.

The available information on the potential chronic effects of heptachlor to aquatic organisms apparently is limited to

a report by Andrews et al. (1966) describing studies in which bluegills were exposed to heptachlor in water and food for 56 days and effects of exposure on growth, survival and cell structure were observed.

Endosulfan (6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) is an organochlorine insecticide for which little information on distribution and persistence in aquatic ecosystems exists. Greve and Wit (1971) reported that endosulfan was present at a concentration of 70 µg/l in water sampled from the Rhine River after a massive fish kill. Information on the toxicity of endosulfan to aquatic organisms is limited to the assessment of acute toxicity and generally indicates that endosulfan is one of the most toxic of these types of chemicals to aquatic organisms. Sanders and Cope (1968) reported the 96-hour LC50 for stonefly naiads to be 2.3(1.6-4.3) µg/l. Pickering and Henderson (1966) reported the 96-hour LC50 of endosulfan to fishes to be ca 3 µg/l. Schoettger (1970) reported the 96-hour LC50 for rainbow trout and white suckers tested at 10°C to be 0.3 µg/l and 2.5 µg/l, respectively. He also reported the 48-hour LC50 for Daphnia magna tested at 19°C was 68 µg/l.

Trifluralin (α,α,α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) is a herbicide which has had widespread use in agriculture. Although little information on distribution and persistence in aquatic ecosystems is available, its acute toxicity to aquatic organisms has been investigated extensively. Sanders (1970) has reported the following 48-hour LC50 values for aquatic invertebrates and trifluralin: Daphnia magna, 560 µg/l; the seed shrimp Cypridopis vidua, 250 µg/l; the scud Gammarus fasciatus, 1800 µg/l; the sowbug Asellus brevicaudus, 2000 µg/l; the grass shrimp Palaemonetes kadiakensis, 1200 µg/l; and the crayfish Orconectes sp., 50,000 µg/l. Similar ranges of toxicity have been reported for other aquatic invertebrates (Sanders and Cope, 1966; 1968). Macek et al. (1969) reported the 96-hour LC50 (95% confidence limits) for trifluralin and bluegills was 190(160-230) µg/l at 12.7°C and 47(40-55) µg/l at 23.8°C. These investigators reported that comparable values for rainbow trout were 210(182-240) µg/l at 1.6°C and 42(38-46) µg/l at 12.7°C. Parks and Worth (1965) reported 96-hour LC50 value for trifluralin and bluegills was 58 µg/l, that for fathead minnows was 93 µg/l, and that for goldfish was 585 µg/l. Information on the effects of longer exposure of aquatic organisms to trifluralin appears to be limited to the studies of Lawrence (1966). He reported that fathead minnows exposed in plastic pools containing soil treated with trifluralin at rates up to 16 lbs./acre exhibited no mortality during four weeks observation.

In view of the lack of definitive information on the potential chronic toxicity of these four pesticides to aquatic organisms, an investigation was designed to estimate the MATC for each chemical and Daphnia magna and fathead minnows. A reason for selecting both an invertebrate and a fish is that susceptibility of fishes to a chemical should be compared to the susceptibility of fish food organisms to that same chemical. It was recently reported (Macek et al., 1976a) that populations of essential fish food organisms may be significantly reduced by chronic exposure to concentrations of lindane which would apparently not directly affect fishes. Therefore, an understanding of the susceptibility of both fishes and fish food organisms to chemicals is necessary in establishing realistic and meaningful water quality criteria and standards.

SECTION II

CONCLUSIONS

Fathead minnows were equally or more sensitive than were daphnids in both acute and chronic exposures to the four pesticides tested. Acute toxicity estimates (LC50's) for daphnids and fathead minnows exposed to acrolein were 57 and 84 µg/l, respectively; heptachlor, 78 and 7 µg/l; endosulfan, 166 and 0.86 µg/l; trifluralin, 193 and 115 µg/l. MATC's estimated from the results of chronic exposures of daphnids and fathead minnows to acrolein were nearly identical for these two species (>16.9 <33.6 µg/l and >11.4 <41.7 µg/l, respectively). MATC's estimated for trifluralin were also similar for both species (>2.4 <7.2 µg/l and >1.9 <5.1 µg/l for daphnids and fathead minnows, respectively). However, for heptachlor and endosulfan, there was an order of magnitude difference between MATC's estimated for daphnids and those for fathead minnows, with the fish appearing to be more sensitve than the daphnids to these two pesticides.

Residues of heptachlor and its epoxide measured in the carcasses of fathead minnows indicated that this pesticide significantly bioconcentrated (20,000X) in fish tissues as a result of aqueous exposure. Trifluralin, on the other hand, exhibited bioconcentration factors by less an order of magnitude than those calculated for heptachlor and fathead minnows. For both pesticides, bioconcentration of residues by fathead minnows was directly proportional to exposure concentrations and was linear over the range of concentrations tested.

SECTION III

RECOMMENDATIONS

Results of the present study indicate that an application factor of 0.01 is appropriate for D. magna exposed to trifluralin. In general, water quality criteria for pesticides are established "by multiplying the acute toxicity values for the more sensitve species... by an application factor of 0.01 except where an experimentallly derived application factor is indicated" (N.A.S., N.A.E., 1973). When calculations are made from the data of the present study the theoretical results for these three exposures correspond reasonably well to the empirically-derived MATC limits. That is, for D. magna, if the 48-hour LC50 of 166 µg/l of endosulfan is multiplied by 0.01, the resulting recommended maximum concentration of endosulfan in water is estimated to be 1.66 µg/l, a concentration which compares favorably with the experimentally determined MATC limits of >2.7<7.0 µg/l. Similarly, with theoretical maximum concentration of trifluralin with respect to D. magna of 1.93 µg/l corresponds to determined MATC limits of >2.4<7.2 µg/l. For P. promelas, these two estimates are 1.15 and >1.9<5.1 µg/l, respectively.

Exposures of both species to acrolein and heptachlor and of fathead minows to endosulfan resulted in calculated application factors which were an order of magnitude higher (0.1) than those utilized to establish water quality criteria (0.01). Therefore, use of the established application factor of 0.01 would result in maximum allowable concentrations of these pesticides in water which should not adversely affect these aquatic organisms.

With regard to the protocol used in the performance of chronic exposures with fathead minnows based on observations made during this study, several recommendations follow:

A reduced level of spawning activity was observed among several groups of fathead minnows in which the number of males was approximately equal to or greater than the number of females. Conversely, a high level of spawning activity was observed among groups of fathead minnows in which survival was reduced and only a few fish remained. Based on these observations we suggest that the number of mature fathead minnows in the spawning chamber be reduced to two males and four females per chamber.

We also feel that adjusting photoperiod to follow Evansville daylengths tends to retard early sexually maturing fathead minnows and we recommend that a constant 16 hour light, 8 hour dark photoperiod be used during the entire chronic exposure of fathead minnows.

Due to poor survival rate which we observed among several fry groups as well as extreme variability in size of fish in the same group, we recommend reducing the number of fathead minnow fry from a total of forty per growth chamber to twenty per growth chamber.

SECTION IV

MATERIALS AND METHODS

Acute bioassay procedures were generally those recommended in Standard Methods for the Examination of Water and Wastewater (APHA 1971). Chronic testing procedures for Daphnia magna were determined through communications between Bionomics staff members and personnel at the National Water Quality Laboratory. The methodology for chronic testing of fathead minnows generally followed the recommended bioassay procedure issued by the Environmental Protection Agency's National Water Quality Laboratory, Duluth, Minnesota (Bioassay Committee, 1971).

EXPOSURE SYSTEMS

Proportional diluters (Mount and Brungs, 1967), with a dilution factor of 0.5 and a syringe injector, delivered the test water and toxicant to the mixing chamber, mixing cells, and ultimately to the test chambers. Five concentrations of toxicant and a control flowed to mixing containers and into separate glass delivery tubes leading to the replicate test chambers.

In the test systems for Daphnia magna, glass battery jars, 17 cm tall and 13.5 cm in diameter, served as the exposure aquaria and baffles were inserted in each to minimize turbulence from influent water. Water depth in each aquarium was 14 cm at which height water passed through a screened outlet. Total volume of exposure solution was 2.0 liters. Quadruplicate aquaria were provided for each toxicant concentration and the diluter was modified to include food cells which delivered a measured amount of food along with the toxicant and diluent water.

Duplicate glass aquaria 30.5 x 90 x 30.5 cm tall were provided for each toxicant concentration in the fathead minnow exposures. Water depth in each aquarium was 15 cm allowing a total volume of 41 liters and the flow rate to each aquarium was equal to 7 tank volumes per 24 hours. Each aquarium was subdivided to provide space for two larval growth chambers 25.0 x 20.5 x 12.5 cm, with a water depth of 15 cm. A 40 mesh stainless steel screen was affixed to one end of each growth chamber to allow water to flow out while retaining the young fish. Test water was delivered directly to the test chamber and growth chambers through a glass, flow-splitting chamber calibrated to keep the flow rate equal in all chambers.

Diluent water was pumped from a 400 foot subterranean well to a cement holding tank. Results of the chemical analysis of the diluent water are summarized in Table 1.

TABLE 1. CHEMICAL ANALYSIS OF THE DILUENT WATER UTILIZED DURING CHRONIC EXPOSURES OF *Daphnia magna* AND FATHEAD MINNOWS TO ACROLEIN, HEPTACHLOR, ENDOSULFAN AND TRIFLURALIN

Parameter	Concentration (mg/l)	Parameter	Concentration (mg/l)
Calcium	6.0	Chloride	17.6
Magnesium	2.1	Fluoride	0.5
Potassium	1.1	Cyanide	<0.005
Sulfate	11.6	Iron	<0.01
Nitrate	<0.05	Copper	0.004
Nitrite	<0.05	Zinc	0.01
Ammonia	0.01	Cadmium	0.001
Phenol	<0.001	Chromium	<0.001
Chlorine	<0.01	Lead	<0.01

Diluent water was delivered through PVC pipes to the exposure systems. Test chambers in the fathead minnow units were maintained in recirculating water baths. Water circulating in the water baths was heated by two immersion coil heaters controlled by a mercury column thermoregulator which maintained a temperature of 25 ± 1°C. Prior to entering experimental units for *Daphnia*, diluent water was delivered to a stainless steel headbox where immersion coil heaters and thermoregulators maintained a temperature of 19 ± 1°C. In addition, ultraviolet lights (24 watt) were placed over the headbox and water cells of each diluter to minimize the introduction of fungus and pathogens into the test system.

Illumination was provided by a combination of Durotest (Optima FS) and wide spectrum Grow Lux fluorescent lights located centrally above the test chambers in all experimental units. A constant photoperiod was controlled by an automatic timer in the *Daphnia* experiments. The photoperiod for fathead minnows followed the normal daylight hours of Evansville, Indiana (average U.S. daylength) starting with the daylength for December 1st (Evansville) on day one of each experiment. Photoperiod was adjusted the first and fifteenth of each month. Experimental units used in all exposures were screened with

black polyethylene curtains to prevent unnecessary disturbance of test organisms and the influence of extraneous lighting on the intended photoperiod.

ACUTE TOXICITY PROCEDURES

Static acute toxicity bioassays were conducted with Daphnia magna and the four pesticides to estimate the 48-hour LC50 and its 95% confidence interval. Five daphnids <24 hours old in each of four replicate containers of each concentration were exposed at 20 ± 1°C. A linear regression equation was calculated after converting test concentrations and corresponding percent mortalities into logarithms and probits, respectively, and this equation was utilized to estimate the 48-hour LC50's and confidence intervals.

Acute toxicity studies with fathead minnows and the four pesticides were conducted in intermittent-flow water systems using proportional diluters (Mount and Brungs, 1967). The incipient LC50 was estimated when no additional significant mortality (<10%) of the test organisms was observed at any concentration during a 48-hour period. At this time, a linear regression equation was calculated by converting test concentrations and corresponding mortalities into logarithms and probits, respectively. This equation was utilized to estimate the incipient LC50 and 95% confidence interval.

The chronic exposure concentrations for both Daphnia and fathead minnows were selected by evaluating observed mortality and no-effect concentrations from the acute studies. The 48-hour LC50 for Daphnia and the incipient LC50 for fathead minnows were used to estimate application factors describing the relationship between acute and chronic toxicity.

Initially, pesticide concentrations and water quality parameters were monitored in each aquarium each week to establish that the concentrations and water quality characteristics were constant with minimum variability. After the determination of the actual concentrations and characteristics in the experimental systems a minimum monitoring effort was conducted to measure variability and detect changes in established means. Stock solutions of heptachlor, endosulfan, and trifluralin (98%, 99%, and 97% a.i., respectively) in Nanograde acetone were delivered to the dilution water from 50 ml glass syringes with stainless steel needles. The solvent (acetone) was not added to the control water in any of the chronic exposures and the amount added to the highest pesticide concentrations did not exceed 43 mg/l in the Daphnia exposures and 12 mg/l in the fathead minnow exposures. Stock solutions of acrolein (99% a.i.) in 100% ethanol were prepared prior to the filling of each 10 ml glass syringe which delivered the solution to the

dilution water. The solvent (ethanol) was not added to the control water and the amount added to the highest acrolein concentration was 3.6 mg/l in the Daphnia exposure and 1 mg/l in the fathead minnow exposure. Generally, pesticide concentrations were determined once each week during each of the chronic exposures by taking water samples from one replicate of each concentration.

CHEMICAL METHODOLOGY

During the chronic exposures, total hardness, alkalinity, pH and acidity were generally measured bi-weekly in the control and one pesticide concentration according to Standard Methods for the Examination of Water and Wastewater (APHA, 1971). Temperature and dissolved oxygen concentrations were measured in selected tanks each day using a YSI dissolved oxygen meter with a combined oxygen-temperature probe.

Acrolein (IUC name: propenal, C_3H_4O) in water was analyzed by adding 2,4-dinitrophenylhydrazine (2,4-DNPH) to form the acrolein:hydrazone complex, extracting the colored complex with benzene and measuring the color intensity spectrophotometrically at 365 nm. The method was modified from Battelle (1970).

A solution of 2,4-dinitrophenylhydrazine (2,4-DNPH), ACS grade, was prepared by adding about 500 ml of distilled water to 167 ml of concentrated hydrochloric acid in a 1000 ml volumetric flask. To this mixture we added 0.792 g of 2,4-DNPH, diluted to 1000 ml with distilled water and stirred until the solids were dissolved. The solution was stored in darkness and fresh solutions were prepared weekly. Amber-glass bottles (3.7 liters), equipped with Teflon-lined caps, were prepared for sample collection by washing the bottles with laboratory detergent, rinsing with tap and then distilled water. The bottles were rinsed twice with 30 ml of benzene, once with methanol, and finally twice with distilled water.

Immediately before sampling, 40 ml of 2,4-DNPH reagent was added to each bottle. Exactly 2000 ml of water was added to each sample bottle, the bottle was capped and swirled to mix the solution. After 1 hour, 900 ml of sample was added to each of two 1-liter separatory funnels equipped with Teflon stopcocks. Each portion was extracted with 25 ml of benzene, followed by a 15 ml benzene extraction, allowing sufficient time after shaking (ca 5-10 min.) for

phase separation. The benzene (ca 80 ml) from the 1800 ml water extraction was combined in a 500 ml separatory funnel (with Teflon stopcock) and extracted twice with 100 ml portions of saturated NaCl, discarding the wash. The benzene was then extracted once with 100 ml of sodium hydroxide (3N), shaking for 15 seconds. A longer extraction time was unnecessary and resulted in emulsions which were difficult to break. The sodium hydroxide wash was discarded and the benzene was passed through a 10 mm I.D. column containing about 50 mm of anhydrous sodium sulfate. The column eluate was collected in a 100 ml volumetric flask. The extracts were diluted to 100 ml with benzene and the absorbance measured at 365 nm utilizing a Coleman Model 6/35 UV/VIS/NIR spectrophotometer with 25 mm cuvettes.

A blank was analyzed concurrently with each batch of aquaria water samples and the blank absorbance was subtracted from each sample absorbance reading to obtain the corrected absorbance used to calculate the acrolein concentration. The blanks used to correct various acrolein concentrations were as follows:

Sample to be analyzed	Blank used for correction
Well water supply	Reagent blank using distilled water
Acrolein diluter delivery tube samples	Well water supply at the diluter location
Acrolein aquaria samples	Acrolein control aquaria samples

On July 12, 1973, the well water supply was analyzed for any substance (aldehyde or ketone) which might react with 2,4-DNPH to present a positive interference in the colorimetric determination of acrolein. The analysis was conducted by the method of additions, namely by analyzing well water containing 0, 8.4, 42, and 105 ng/liter of added acrolein. The results show that the well water contained 9 ng/liter of interfering compounds, calculated as acrolein. This concentration of contaminant remained constant throughout the study as documented by well water analyses performed concurrently with each batch of diluter water samples.

The data presented have been corrected for the observed background. All acrolein results were calculated from a standard graph of ppb acrolein versus absorbance at 365 nm. Data generated from the following standards showed only mild deviation from Beer's Law. The minimum concentration detectable by this method was 3 ng/liter.

µg/l Acrolein	Absorbance
4.0	0.050
8.4	0.105
42.0	0.396
105.0	0.766

Heptachlor (IUC name: 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-endo-methanoindene) and its degradation product, heptachlor epoxide (IUC name: 1,4,5,6,7,8,8-Heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan) were analyzed from water and fish tissue by gas/liquid chromatography using electron capture detection.

Water samples (400-500 ml) were collected in amber glass bottles, equipped with Teflon-lined caps. The entire content of the sample bottle was measured and added to a 500 ml separatory funnel, equipped with a Teflon stopcock. Then ca 50 ml of nanograde methylene chloride was added to the bottle, the cap replaced, and the bottle shaken to extract any pesticide which may have been adsorbed onto the glass surface from the aqueous phase. The solvent was transferred quantitatively from the bottle to the separatory funnel and the water sample was extracted with this solvent portion. After phase separation, the methylene chloride was drained into a 250 ml Griffin beaker. The water was extracted a second time with 30 ml of methylene chloride, which was added to the first portion of solvent.

The combined solvent was passed through a 20 mm I.D. glass column, equipped with a glass-frit and containing 50 mm of anhydrous sodium sulfate. When the initial solvent had almost reached the top surface of the sodium sulfate, an additional portion of fresh methylene chloride (ca 30 ml) was added to rinse any pesticide residue from the column walls and the sodium sulfate. The solvent was added to a 500 ml Kuderna-Danish evaporator as it dripped from the sodium sulfate column. The evaporator was lowered and the tip of the column was rinsed (with fresh solvent) into the evaporator. The Kuderna-Danish evaporator was fitted with a three-ball Snyder column and the solvent was concentrated to ca 3 ml over a steam bath at 70°C. The evaporator was cooled to room temperature and the solvent was transferred to a 15 ml centrifuge tube (Pyrex 8064 or equivalent), rinsing with fresh methylene chloride. The remaining solvent was evaporated to dryness at room temperature, using a steam of clean dry air. Immediately after the last trace of methylene chloride had evaporated, a known volume (1-10 ml) of Nanograde hexane was added to dissolve the residue and an aliquot was withdrawn for analysis of heptachlor and its

epoxide by gas chromatography.

The gas chromatographic operating conditions were:

Instrument: Tracor Model MT-550 Dual Column
Detector: 15mC Ni^{63} electron capture
Column: 2 m x 2 mm I.D. glass column packed with 80/100 mesh Supelcoport coated with 5% (by weight) of DC-200. The column support was coated according to the fluidized drying technique of Kruppa et al. (1967), using a packing dryer supplied by the CRC Co., Addison, Ill.
Gas flow: 35 cc/minute
Column: 35 cc nitrogen/minute at 27 psi column pressure
Detector: 60 cc nitrogen/minute
Temperature:
Inlet: 215°C Outlet: 210°C
Column: 190°C Detector: 290°C
Recorder: Corning Model 841, 0-1 mv full-scale
Response: $1x10^{-10}$g of heptachlor and $5x10^{-10}$g of heptachlor epoxide gave half-scale recorder pen response using an electrometer attenuation of $1.6x10^{-9}$ amperes.

Prior to the analysis of each batch of water samples, ca 100 ug of heptachlor and the epoxide were injected to saturate the column. After a stable baseline was obtained (ca 1 hr.) a series of heptachlor and heptachlor epoxide standards (Chem Service Cat#78 and 700, respectively) was chromatographed and a graph was constructed of nanograms injected versus the recorder response in millimeters of peak height. The nanograms of pesticide represented by sample peak heights were read from the graph and the results were calculated. A calibration graph was constructed prior to the analysis of each batch of heptachlor water samples; furthermore, during the period of chromatographic analysis, standard solutions of known weight were analyzed after every ten sample extracts to verify the stability of instrument response throughout the day.

Quality control samples were manufactured with well water which contained known weights of heptachlor and heptachlor epoxide, added to the water using a small volume (0.1-0.5 ml) of Nanograde acetone as a co-solvent. The results of the percentage recoveries and standard deviations obtained (Table 2) show that no loss of the two compounds occurred during sample preparation.

TABLE 2. RECOVERY OF HEPTACHLOR AND HEPTACHLOR EPOXIDE FROM QUALITY CONTROL WATER SAMPLES

Conc. added (µg/l)		Water volume		Percentage recovery	
H.[a]	H.E.[b]	(ml)		H.	H.E.
1.0	1.0	400		96	98
				103	96
				98	106
				97	103
				100	95
			(Average)	99 ± 2.6[c]	100 ± 4.7
10	10	400		102	106
				94	96
				99	102
				96	94
				105	98
			(Average)	99 ± 4.3	99 ± 4.8

[a]H. denotes heptachlor

[b]H.E. denotes heptachlor epoxide

[c]Standard deviation

Fish tissues were extracted for heptachlor and heptachlor epoxide residues using a column technique modified from a procedure described by Hesselberg and Johnson (1972). These authors ground fish tissue in a Waring Blendor along with dry ice, stored the tissue at -20°C for 8-12 hours, and then proceeded with the solvent extraction step. E G & G, Bionomics used Tekmar analytical mill, Model A-10, featuring internal metal tubing which circulates a liquid (from an external unit) around the grinding chamber to raise or lower the temperature.

Sodium sulfate was purified by heating it for 16 hours at 450°C, cooling, and storing the compound in clean, all-glass bottles. Anhydrous sodium sulfate (ca 5 times the tissue weight) was added to the grinding chamber, followed by 2.0 grams of Celite 545, and finally the fish tissue. Coolant was circulated through the mill until the temperature was minus 50°C, and the tissue was homogenized by grinding at 20,000 rpm for 30 seconds. The resultant fine powder was quantitatively transferred to a chromatographic column (20 x 400 mm) and eluted with 250 ml of 50 percent ethyl ether in petroleum ether. The eluate was collected (1-2

ml/min.) in a 500 ml Kuderna-Danish evaporator, then a three-ball Snyder column was installed and the solvent was evaporated to a volume of ca 2-4 ml over a water bath at 70°C.

A large portion of extraneous materials, e.g., lipids, fats, and oils, were removed from the tissue extract by performing the hexane-acetonitrile partition clean-up procedure described by the U.S. Department of the Interior (1972). The resultant acetonitrile solution of the clean tissue extract was placed in a Kuderna-Danish evaporator, equipped with a three-ball Snyder column and concentrated to ca 3 ml over a steam bath. The evaporator was cooled to ambient temperature and the solvent was quantitatively transferred to a 15 ml graduated centrifuge tube equipped with a metal-lined screw cap. The solvent was adjusted to the volume required for analysis by evaporating the solvent at ambient temperature, using a stream of clean dry air, and then diluting to volume with Nanograde hexane.

An aliquot of the extract was analyzed by gas chromatography with the following instrumental conditions:

Instrument: Tracor Model MT-550
Detector: 15 mC Ni^{63} electron capture
Column: 2 m x 2 mm ID glass containing 5% Dexsil 300 GC on 80/100 mesh Supelcoport packing. The column was prepared according to the fluidized drying method of Kruppa et al. (1967).
Gas Flows: 35 cc/min.
Detector: 60 cc He/min.
Column: 30 cc He/min at 18 psi back pressure
Recorder: Corning Model 841, 0-1 mV full-scale
Response: 1×10^{-10}g of heptachlor and 2×10^{-10}g of heptachlor epoxide eluted in 4.85 and 9.77 minutes, respectively; and gave half-scale recorder pen deflection at an electrometer attenuation of 1.6×10^{-9} amperes.

The Dexsil 300 GC column was chosen for its extreme stability at high temperatures. After analyzing ca ten tissue extracts, the column temperature was elevated to 300°C overnight. The next morning a large amount of heptachlor and heptachlor epoxide (100 µg) was injected and the column temperature was reduced to 210°C. Several injections of identical weights (ca 0.1 ng) of the two standard compounds were made and identical peak heights for all injections was assumed to indicate column saturation. At this point the analysis of another batch of tissue extracts proceeded. In this manner, the need for more exhaustive clean-up of tissue was negated and a greater number of tissue extracts could be chromatographed with no apparent change in sensitivity or retention time of heptachlor or heptachlor epoxide.

Table 3 lists the percentage recovery and standard deviation of heptachlor and heptachlor epoxide from quality control samples using fathead minnow tissue known to be free from naturally-occurring residues of either compound. According to the U.S. Department of the Interior (1972), the tissue extract was dissolved in 30 ml of hexane (saturated with acetonitrile) and extracted twice with two 30 ml portions of acetonitrile (saturated with hexane). The combined acetonitrile extracts were evaporated, as previously described, and the extract was dissolved in hexane. The low percentage recovery of both compounds was assumed to be due to the above clean-up procedure, in that: 1) only two extractions of the hexane phase were specified by this method; and 2) evaporation of the acetonitrile (b.p. 81.6°C) may cause some loss of these compounds.

Table 3. RECOVERY OF HEPTACHLOR AND HEPTACHLOR EPOXIDE FROM QUALITY CONTROL TISSUE SAMPLES[a]

Weight added (µg) H.[b]	Weight added (µg) H.E.[c]	Tissue Weight (g)		Percentage recovery H.	Percentage recovery H.E.
1.0	1.0	1.776		38	39
1.0	1.0	1.674		40	30
1.0	1.0	1.776		38	30
1.0	1.0	1.758		36	29
			Average	38±1.6[d]	32±4.7

[a]Quality control samples were manufactured using fathead minnows fillets

[b]H. denotes heptachlor

[c]H.E. denotes heptachlor epoxide

[d]Standard deviation

The data were calculated using standard graphs as previously described for the analysis of heptachlor and its epoxide from water. The calibration graphs were also prepared prior to analyzing a particular batch of tissue samples. Results of analysis of fish tissues are corrected for recovery.

Endosulfan (IUC name: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) was analyzed from water by the extraction procedure and gas

chromatographic analysis previously described for heptachlor. Standard reference material was supplied by the Niagara Chemical Division, FMC Corp. Middleport, N.Y. and was identified as follows:

Compound	Batch No.	Purity %
Thiodan ether	D188-87	100
Thiodan alcohol	D188-85	100
Thiodan I[a]	ME-J264	99.9
	ME-J264	99.9
Thiodan II[b]	ME-J265	95.5
	ME-J265	95.5
Thiodan sulfate	MR-C747	100

[a]Numeral (I) denotes the lower melting (α) isomer

[b]Numeral (II) denotes the higher melting (β) isomer.

The retention times of the various endosulfans using a column temperature of 215°C were:

	RTA[a]	Response (nanograms)[b]
Endosulfan ether	0.62	1.0
Endosulfan alcohol	1.31	0.6
Endosulfan I	1.60	0.1
Endosulfan II	2.20	0.2
Endosulfan sulfate	2.93	4.0

[a]RTA: retention time relative to standard aldrin designated as 1.00. Aldrin eluted in 4.20 minutes.

[b]Nanograms of compound which produced half-scale recorder response at an electrometer attenuation of 1.6×10^{-9} amperes.

In all cases, two liters of water were extracted with Nanograde methylene chloride, the extract was concentrated using Kuderna-Danish units, and the last trace of solvent was removed with a gentle air current. The extract was immediately dissolved in Nanograde hexane (0.5-5 ml) and an aliquot was analyzed by gas chromatography. The minimum detectable concentration was 0.06, 0.04, 0.006, 0.012, and 0.2 µg/l for endosulfan ether, alcohol, isomer I, isomer II, and the sulfate, respectively. The percentage recoveries of endosulfan I and II are listed in Table 4.

The endosulfan used to manufacture diluter stock solutions during the chronic toxicity test was examined to determine the percentages of isomers I and II by dissolving a known

amount of the compound in hexane and analyzing the solution by gas chromatography. The compound was determined to be 100 percent active containing 60.0 and 40.0 percent of isomers I and II, respectively.

TABLE 4. RECOVERY OF ENDOSULFAN FROM QUALITY CONTROL WATER SAMPLES

Concentration added (µg/l)	Water volume (ml)	Percentage recovery
0.025	2000	92
		108
		104
		100
		96
		Average 100 ± 6.3[a]
0.125	2000	87
		100
		96
		93
		94
		Average 94 ± 4.7

[a]Standard deviation

On several test days (random), namely days 0, 5, 107; the aquaria water from the highest endosulfan exposure (0.6 µg/l) was examined for the presence of degradation products. Endosulfan ether, alcohol, and sulfate were not detected in any of the water samples analyzed.

Endosulfan containing isomers I and II at a 60:40 ratio was used to manufacture the quality control samples in well water. The analysis of these samples revealed the same ratio of isomers present on the chromatogram, indicating no difference in the percentage recovery of the two isomers. The recovery of endosulfan was considered to be complete and no correction factor was applied to the calculation of results. Peak heights of endosulfan I and II extracted from water were compared to analytically pure endosulfan standards containing known weights and ratios of isomers I and II. The concentrations of isomers I and II were summed and the data were presented as total endosulfan.

Trifluralin (α,α,α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) was analyzed from water and fish tissue by the methodology previously described for heptachlor. Technical trifluralin, (Lot No. 5GB70, 99 percent active, from Eli-Lilly and Co., Indianapolis, Ind.) was used to calibrate the gas chromatograph prior to and during the analysis of trifluralin samples. Using an electrometer attenuation of 1.6×10^{-9} amperes, 0.05 nanograms of trifluralin produced half scale recorder response, eluting from the column in 1.80 minutes. The extreme response of trifluralin to electron capture detection enabled a minimum detectable concentration of 0.08 µg/liter, when extracting a 65 ml volume of water. The minimum detectable concentration of trifluralin from fish tissue, due to the low percentage recovery and the interference of fats and oils which survive the clean-up procedure was 0.10 µg/gm. The percentage recovery of trifluralin from well water is listed in Table 5 and from fish tissue in Table 6. Reported concentrations of trifluralin in water and fish were calculated by multiplying the raw data by factors of 1.20 and 5.55, respectively.

TABLE 5. RECOVERY OF TRIFLURALIN FROM QUALITY CONTROL WATER SAMPLES

Concentration added (µg/l)	Water volume (ml)	Percentage recovery
0.025	400	84
		84
		76
		81
		75
		Average 80 ± 4.3[a]
0.125	65	78
		87
		85
		77
		88
		Average 83 ± 5.1

[a]Standard deviation

TABLE 6. RECOVERY OF TRIFLURALIN FROM QUALITY CONTROL TISSUE[a] SAMPLES

Concentration added (µg/gm)	Tissue weight (g)	Percentage recovery
0.563	1.776	18
0.597	1.674	21
0.678	1.476	17
0.569	1.758	19
0.651	1.536	15
		Average 18 ± 2.2[b]

[a]Whole fathead minnows

[b]Standard deviation

STATISTICS

Measured biological parameters from replicate containers during chronic exposure were averaged and subjected to analysis of variance according to Steel and Torrie (1960). When treatment effects were indicated, the means of these effects were subjected to Duncan's Multiple Range Test to determine which treatments were statistically different. All differences were considered statistically significant at a probability of P=.05.

CHRONIC EXPOSURE

Daphnia magna

Laboratory stocks of Daphnia magna were obtained from the University of New Hampshire, Durham, N.H., and successfully cultured in the laboratory according to the methods of Biesinger and Christensen (1972). Typically, 10 daphnids (<24 hours old) were placed into each of four replicate experimental units, resulting in a total of 40 animals per concentration. A food supply consisting of trout starter and dry powdered cerophyl (2:1) was prepared in an aqueous suspension (12.5 mg/ml) and delivered from a Marriotte bottle via a volumetric delivery system to a mixing chamber during each diluter cycle. The diluted food suspension was subsequently transferred to the food cells from which 25 ml (0.1 mg/ml) were delivered to each test container during each diluter cycle.

Survival and reproduction of Daphnia were recorded after one, two, and three weeks. Reproduction was measured by recording the number of young in each experimental chamber weekly and discarding the progeny after weeks one and two. At the end of the third week, the number of original animals remaining was recorded, the specimens discarded, and 10 daphnids were randomly selected from each chamber to begin the second generation exposure. The same procedures were followed for the second and third generation, after which the experiment was terminated.

Pimephales promelas

Chronic exposures of fathead minnows with endosulfan and trifluralin began in September 1972, with heptachlor in April 1973, and with acrolein in June 1973. Fathead minnows, obtained as eggs from the Newtown Fish Toxicology Laboratory, Newtown, Ohio, were 20 and 26 days old on day one of the endosulfan and trifluralin chronic exposures, respectively. Fathead minnows from brood stock at the Aquatic Toxicology Laboratory of Bionomics, EG&G, Inc., Wareham, Massachusetts were used to initiate chronic exposures with heptachlor and acrolein. Test fish were one day old for heptachlor and 27 days old for the acrolein chronic exposure. In each test, forty fish were randomly distributed to each test chamber. Cumulative mortality and total length of live fish were determined after 30 and 60 days using the photographic method of McKim and Benoit (1971). After 60 days exposure the number of fish in each test chamber was impartially reduced to fifteen. Fathead minnows were fed ad libitum twice daily with a commercially prepared trout starter food which was supplemented with daphnids and brine shrimp nauplii. All tanks were siphoned twice weekly to remove fecal material, excess food, and detritus and were brushed when algal growth became excessive.

The discovery of external parasites on a few fish in the trifluralin and endosulfan chronic exposures prompted the use of flush treatments with a combination of malachite green and formalin (25 µl/liter of formalin containing 3.7 g/l malachite green crystals). These treatments were concentrated between days 115 and 130 in each of the chronic exposures and all tanks were treated equally whether or not parasites were found.

Due to unequal numbers of males in each tank, some of the male fish were removed after secondary sexual characteristics were well developed. Five spawning sites of halved, 3 inch transite drain tiles had been placed in each tank when fish

were released from growth chambers after 60 days. The tiles were placed concave surface down at locations that minimized the chance of encounters by separate egg-guarding males. When spawning began, eggs were removed and counted after 1:00 P.M. each day. Fifty eggs from each spawn were oscillated in their corresponding test water by means of an egg cup and a rocker arm apparatus (Mount, 1968). Dead eggs were removed and counted each day until hatching was completed (3-5 days at 20°C). Percent hatch was based on the number of live fry from 50 eggs.

Forty fry from the earliest two spawns in each tank with at least 80% live hatch were placed in the respective growth chambers. Cumulative mortality and total length of live fish were determined at 30 and 60 days photographically (McKim and Benoit, 1971). Fry from all other spawns were discarded unless a growth chamber was later made available by termination of 60 day old fry. Finely ground starter food and brine shrimp nauplii were fed three times daily to fry in the growth chambers.

Parental fish were sacrificed after all spawning had ceased for one week. Total length, weight, sex and gonadal condition was determined for each fish and three samples of eviscerated fish per concentration were retained for residue analysis in the tests with trifluralin and heptachlor.

SECTION V

RESULTS

ACUTE BIOASSAYS

The analysis of the results of acute static bioassays with Daphnia magna indicated the 48-hour LC50 (95% confidence interval) was 57 (20-99) µg/l for acrolein, 78 (46-113) µg/l for heptachlor, 166 (126-219) µg/l for endosulfan, and 193 (115-327) µg/l for trifluralin. Toxicity induced mortality was observed with daphnids after 48-hour exposure to 50 µg/l of acrolein, 50 µg/l of heptachlor, 150 µg/l of endosulfan and 100 µg/l of trifluralin. Based on these data, the highest nominal concentration selected for investigation during chronic exposure was 60 µg/l of acrolein, 50 µg/l of heptachlor, 100 µg/l of endosulfan and 100 µg/l of trifluralin.

A 6 day continuous-flow bioassay conducted at 25 + 1°C with 51 day old fathead minnows indicated the incipient LC50 of acrolein to this species was 84 (54-130) µg/l. A 10 day continuous-flow bioassay conducted at 25 + 1°C with 60 day old fathead minnows resulted in an estimated incipient LC50 of 7.0 (5.7-8.5) µg/l of heptachlor. The incipient LC50 of endosulfan for fathead minnows was estimated to be 0.86 (0.52-1.4) µg/l based on a 7 day continuous-flow bioassay with 53 day old fathead minnows. The incipient LC50 of trifluralin for fathead minnows was estimated to be 115 (48-211) µg/l based on a 12 day continuous-flow bioassay with 44 day old fathead minnows. Based on these data, the highest nominal concentration selected for investigation during chronic exposure of fathead minnows was 89 µg/l of acrolein, 4 µg/l of heptachlor, 0.60 µg/l of endosulfan and 20 µg/l of trifluralin.

WATER CHEMISTRY

The results of the chemical analysis of water samples taken during the various chronic exposures indicate that hardness, alkalinity, acidity, dissolved oxygen and pH varied minimally both within any one chronic exposure and between chronics. Statistical analysis showed no significant differences for any of the above parameters between treatments within a chronic; therefore, only means and ranges for the various parameters are presented (Tables 7 and 8).

The results of gas chromatographic analyses of water samples taken periodically during chronic exposure of Daphnia magna to the three of the four pesticides are presented in Table 9. During the heptachlor experiment, contamination of most of the water samples with interfering trace organics in sufficient quantities to preclude the quantitation of heptachlor was encountered. After considerable investigation of test water,

TABLE 7 MEAN AND RANGE OF MEASURED CONCENTRATIONS OF HARDNESS, ALKALINITY, ACIDITY, DISSOLVED OXYGEN AND pH FROM WATER SAMPLES TAKEN PERIODICALLY DURING CHRONIC EXPOSURE OF Daphnia magna TO FOUR PESTICIDES

Pesticide	Concentration (mg/l) Hardness	Alkalinity	Acidity	D.O.[a]	pH
ACROLEIN					
Mean ± S.D.[b]	35 ± 2.1	33 ± 2.1	4.4 ± 0.8	7.5 ± 0.6	-
Range	29 - 38	30 - 34	3.8 - 4.8	6.5 - 8.6	7.0 - 7.3
(N)	(5)	(5)	(5)	(29)	(5)
HEPTACHLOR					
Mean ± S.D.	37 ± 2.5	34 ± 2 6	4.7 ± 1.8	6.9 ± 1.5	-
Range	35 - 42	32 - 39	3.9 - 5.2	4.1 - 8.8	6.8 - 7.2
(N)	(4)	(4)	(4)	(28)	(5)
ENDOSULFAN					
Mean ± S.D.	35 ± 3.1	32 ± 3.6	4.6 ± 1.5	7.0 ± 0.6	-
Range	30 - 39	26 - 35	3.9 - 5.0	6.4 - 8.0	6.8 - 7.1
(N)	(5)	(5)	(5)	(24)	(5)
TRIFLURALIN					
Mean ± S.D.	37 ± 2.3	35 ± 2.8	4.4 ± 0.9	6.6 ± 1.0	-
Range	34 - 39	31 - 37	3.8 - 4.7	4.6 - 8.7	6.8 - 7.2
(N)	(5)	(5)	(5)	(22)	(5)

[a] Dissolved oxygen

[b] Standard deviation

TABLE 8. MEAN AND RANGE OF MEASURED CONCENTRATIONS OF HARDNESS, ALKALINITY, ACIDITY, DISSOLVED OXYGEN AND pH FROM WATER SAMPLES TAKEN PERIODICALLY DURING CHRONIC EXPOSURE OF FATHEAD MINNOWS (Pimephales promelas) TO FOUR PESTICIDES

Pesticide	Concentration (mg/l) Hardness	Alkalinity	Acidity	D.O.[a]	pH
ACROLEIN					
Mean ± S.D.[b]	32 ± 4.7	30 ± 3.7	3.6 ± 1.5	8.2 ± 1.3	-
Range	23 - 39	20 - 35	1.9 - 7.0	4.9 - 9.6	6.6 - 6.8
(N)	(20)	(20)	(20)	(798)	(20)
HEPTACHLOR					
Mean ± S.D.	33 ± 5.1	32 ± 3.8	4.6 ± 1.6	7.5 ± 1.2	-
Range	21 - 40	21 - 36	2.9 - 6.0	4.3 - 9.6	6.6 - 7.0
(N)	(14)	(14)	(16)	(888)	(16)
ENDOSULFAN					
Mean ± S.D.	35 ± 3.4	28 ± 3.4	4.4 ± 1.5	7.9 ± 1.0	-
Range	31 - 40	22 - 31	2.9 - 6.7	4.8 - 9.4	6.7 - 7.3
(N)	(12)	(12)	(12)	(1386)	(12)
TRIFLURALIN					
Mean ± S.D.	33 ± 4.5	27 ± 2.6	4.2 ± 1.5	7.8 ± 1.0	-
Range	23 - 39	23 - 31	2.0 - 7.0	5.9 - 9.5	6.6 - 7.2
(N)	(12)	(12)	(12)	(1356)	(12)

[a]Dissolved oxygen

[b]Standard deviation

TABLE 9. NOMINAL AND MEAN MEASURED CONCENTRATIONS OF ACROLEIN, ENDOSULFAN AND TRIFLURALIN IN WATER DURING CONTINUOUS EXPOSURE OF Daphnia magna

Compound	Nominal Concentration (µg/l)	Measured Concentration (µg/l) Mean ± S.D.[a]	Range	# Samples
ACROLEIN	60	42.7 ± 4.3	41-47	8
	30	33.6 ± 7.8	24-35	8
	15	16.9 ± 4.2	15-21	8
	8	7.1 ± 5.8	6-14	8
	4	3.2 ± 0.7	3-4	8
ENSOSULFAN	100	79.7 ±28.6	47-100	8
	50	37.7 ±12.5	25-50	8
	25	15.3 ± 4.0	13-20	8
	12	7.0 ± 4.4	2-10	8
	6	2.7 ± 2.9	1-6	8
TRIFLURALIN	100	52.7 ±10.7	37-60	6
	50	25.6 ± 4.7	21-32	6
	25	14.0 ± 3.7	10-20	6
	12	7.2 ± 2.0	4-9	6
	6	2.4 ± 1.3	1-4	6

[a]Standard deviation

glassware, extraction solvents, column components, etc. we determined that the batch of acetone solvent utilized to prepare the heptachlor stock solution was contaminated with unknown interfering trace organics. Unable to separate heptachlor from these contaminants by various clean-up techniques, we have reported all data for Daphnia on the basis of nominal concentrations.

The results of gas chromatographic analysis of water samples taken periodically during the chronic exposure of fathead minnows to the four pesticides studies are presented in Table 10. For endosulfan and trifluralin, mean measured concentrations were fairly consistent and varied only slightly from nominal. The mean measured acrolein and heptachlor concentrations were generally less than half of nominal. Although we detected heptachlor epoxide in water samples suggesting degradation in the system, observations of the epoxide were intermittent and sporadic. Rather, we believe the loss of approximately half of the heptachlor in the system was due to adsorption of the chemical into glass, food, and/or fecal material, or to incorporation into fish tissues, or to both factors. During the period from day 210 to the end of

TABLE 10. NOMINAL AND MEAN MEASURED CONCENTRATIONS OF ACROLEIN, HEPTACHLOR, ENDOSULFAN AND TRIFLURALIN DURING CHRONIC EXPOSURE OF FATHEAD MINNOWS (Pimephales promelas)

Compound	Nominal Conc. (µg/l)	Measured concentration (µg/l) Mean ± S.D.[a]	Range	# Samples
ACROLEIN	89	41.7 ± 35.8	9-140	28
	45	20.8 ± 15.5	5-60	12
	22	11.4 ± 8.3	3-32	15
	11	6.4 ± 3.6	2-13	13
	5	4.6 ± 2.7	1-10	15
HEPTACHLOR	4.0	1.84 ± 0.64	1.2-2.3	28
	2.0	0.86 ± 0.29	0.72-1.2	18
	1.0	0.43 ± 0.20	0.29-0.74	28
	0.5	0.20 ± 0.07	0.17-0.27	18
	0.25	0.11 ± 0.03	0.07-0.14	18
ENDOSULFAN	0.60	0.40 ± 0.16	0.21-0.85	58
	0.30	0.20 ± 0.08	0.05-0.39	29
	0.15	0.10 ± 0.03	0.04-0.17	29
	0.08	0.06 ± 0.03	0.02-0.13	28
	0.04	0.04 ± 0.03	0.01-0.12	30
TRIFLURALIN	20.0	16.5 ± 14.0	3.6 - 50	56
	10.0	8.2 ± 6.2	1.9 - 28	28
	5.0	5.1 ± 4.2	1.4 - 18	33
	2.5	1.9 ± 2.1	0.37-5.5	28
	1.2	1.5 ± 1.2	0.40-4.2	30

[a]Standard deviation

the heptachlor fathead minnow chronic we experienced interferences in the analysis of water samples for heptachlor similar to those previously described. Despite significant effort, we were unable to identify the source of these interferences during the chronic. We have subsequently identified contamination of the solvent system as the likely cause of these difficulties. Acrolein is a very unstable compound and is readily oxidized; also it is extremely volatile (boiling point 48°C). Thus it is not surprising that measured concentrations were significantly lower than nominal.

CHRONIC EXPOSURE

Daphnia magna to acrolein

Statistical analysis of data on the survival of Daphnia magna continuously exposed to acrolein for 64 days indicated significant differences due to treatment. During the first generation, continuous exposure to mean measured concentrations of 42.7 and 33.6 µg/l of acrolein for 22 days significantly reduced survival of daphnids (Table 11). During the second generation the effect on survival was cumulative for daphnids exposed to 33.6 µg/l of acrolein. The apparent reduction in survival of second generation Daphnia exposed to 16.9 µg/l of acrolein on day 43, was not consistent with observations on the third generation where survival at this concentration was equal to that of the controls. Production of young during the first generation was extremely variable but generally indicated no significant reductions which could be correlated to chemical treatments (Table 12). Production of young during generations two and three was less variable than during generation one and indicated that no significant differences existed among treatments where reproduction occurred.

Based on the observed effects of acrolein on survival of Daphnia magna continuously exposed through three generations of development, the estimated maximum acceptable toxicant concentration for this species is >16.9 <33.6 µg/l.

Daphnia magna to heptachlor

Although we recognize the contamination of the solvent system could possibly have affected the daphnids, for purposes of discussion all experimental observations which appear dose-related are presumed to be the effect of heptachlor in water. Survival of Daphnia magna continuously exposed to nominal concentrations of 50 and 25 µg/l of heptachlor for 64 days was reduced when compared to controls (Table 13). This effect was observed in all three generations of daphnids exposed to 50 µg/l, but only among the second and third generation exposed to 25 µg/l. Survival among daphnids continuously

TABLE 11. MEAN[a] PERCENT SURVIVAL OF Daphnia magna CONTINUOUSLY EXPOSED TO ACROLEIN FOR 64 DAYS

Mean Measured Conc. (µg/l)	GENERATION[b] I			GENERATION II			GENERATION III		
	DAY			DAY			DAY		
	8	15	22	29	36	43	50	58	64
42.7	93	86	0	-	-	-	-	-	-
33.6	55	53	28	3	2	2	2	2	2
16.9	68	60	60	60	53	35	98	83	78
7.1	95	86	83	75	73	70	100	93	78
3.2	100	75	63	70	60	58	73	70	65
Control	98	93	83	95	83	80	98	88	78

[a]Each value represents the mean of four replicates

[b]Duration of exposure for generations I, II and III were days 1-22, 22-43, 43-64, respectively

TABLE 12. MEAN[a] PRODUCTION OF YOUNG PER FEMALE Daphnia magna CONTINUOUSLY EXPOSED TO ACROLEIN FOR 64 DAYS.

Mean Measured Conc. (µg/l)	GENERATION[b] I		GENERATION II		GENERATION III	
	DAY		DAY		DAY	
	15	22	36	43	57	64
42.7	5	-	-	-	-	-
33.6	1	8	15	29	17	22
16.9	16	21	15	12	17	15
7.1	14	16	14	22	12	14
3.2	2	14	11	15	13	14
Control	10	10	11	20	16	17

[a]Each value represents the mean of four replicates

[b]Duration of exposure for generations I, II, and III were days 1-22, 22-43, 43-64, respectively

TABLE 13. MEAN[a] PERCENT SURVIVAL OF *Daphnia magna* CONTINUOUSLY EXPOSED TO HEPTACHLOR FOR 64 DAYS

Nominal Conc. (µg/l)	GENERATION[b] I			GENERATION II			GENERATION III		
	DAY			DAY			DAY		
	8	15	22	29	36	43	50	57	64
50.0	88	65	28	24	23	15	23	23	13
25.0	100	95	78	94	55	53	68	65	40
12.5	95	93	90	94	88	85	100	95	75
6.25	93	93	88	94	90	65	75	70	48
3.12	100	85	80	62	50	50	75	55	40
Control	100	83	80	93	88	80	95	90	73

[a]Each value represents the mean of four replicates

[b]Duration of exposure for generations I, II and III were days 1-22, 22-43, 43-64, respectively

exposed to 12.5 µg/l heptachlor was not significantly different from controls. Although survival of daphnids exposed to the two lowest concentrations of heptachlor appeared to be reduced, in view of the survival observed among daphnids continuously exposed to 12.5 µg/l we do not consider these observations to be pesticide related (Table 13). Analysis of variance of data related to production of young per female *Daphnia magna* during generations one, two, and three indicates no significant differences due to treatment (Table 14).

TABLE 14. MEAN[a] PRODUCTION OF YOUNG PER FEMALE *Daphnia magna* CONTINUOUSLY EXPOSED TO HEPTACHLOR FOR 64 DAYS

Nominal Conc. (µg/l)	GENERATION[b] I		GENERATION II		GENERATION III	
	DAY		DAY		DAY	
	15	22	36	43	57	64
50.0	24	63	28	26	44	36
25.0	18	44	22	42	41	59
12.5	34	60	31	34	41	52
6.25	30	54	24	46	32	44
3.12	46	42	33	28	40	21
Control	33	52	29	29	41	47

[a]Each value represents the mean of four replicates

[b]Duration of exposure for generations I, II, and III were days 1-22, 22-43, and 43-64, respectively

Based on the observed effects of heptachlor on survival and reproduction of Daphnia magna continuously exposed through three generations, the estimated maximum acceptable toxicant concentration for this species, based on nominal concentrations, is >12.5 <25 µg/l.

Daphnia magna to endosulfan

Statistical analysis of data on survival of Daphnia continuously exposed to endosulfan for 64 days indicated significant differences due to treatment. Exposure to mean measured concentrations of 79.7, 37.7, 15.3 and 7.0 µg/l endosulfan for 22 days, significantly reduced survival of daphnids (Table 15). This effect on survival was cumulative, with survival of second generation daphnids being significantly less than that of the first generation. After one week of exposure of the third generation, effects on survival again appeared to be cumulative at these concentrations and was lower than during the first week of the previous two generations. The poor survival of third generation daphnids in the control and 2.7 µg/l endosulfan treatment after two and three weeks precludes drawing valid conclusions about cumulative effects of exposure to this concentration on survival between generations two and three.

TABLE 15. MEAN[a] PERCENT SURVIVAL OF Daphnia magna CONTINUOUSLY EXPOSED TO ENDOSULFAN FOR 64 DAYS

Mean Measured Conc. (µg/l)	GENERATION[b] I			GENERATION II			GENERATION III		
	DAY			DAY			DAY		
	8	15	22	29	36	43	50	57	64
79.7	93	58	38	83	0	0	0	0	0
37.7	95	48	45	90	8	8	5	5	5
15.3	80	63	30	95	3	3	28	23	23
7.0	85	83	55	73	8	8	28	20	15
2.7	98	98	88	95	90	73	100	8	0
Control	100	100	100	93	90	85	80	44	15

[a]Each value represents the mean of four replicates

[b]Duration of exposure for generations I, II and III were days 1-22, 22-43, 43-64, respectively

Production of young during the first generation of Daphnia exposed to endosulfan indicates no significant differences which can be correlated to toxicant concentration (Table 16).

TABLE 16. MEAN[a] PRODUCTION OF YOUNG PER FEMALE Daphnia magna CONTINUOUSLY EXPOSED TO ENDOSULFAN FOR 64 DAYS

Mean Measured Conc. (µg/l)	GENERATION[b] I		GENERATION II		GENERATION III	
	DAY		DAY		DAY	
	15	22	36	43	57	64
79.7	7	17	-	-	-	-
37.7	7	23	5	2	5	12
15.3	4	15	23	43	14	14
7.0	6	9	18	19	15	21
2.7	13	12	19	24	0[c]	0
Control	8	12	17	34	6	17

[a]Each value represents the mean of four replicates

[b]Duration of exposure for generations I, II, and III were days 1-22, 22-43, 43-64, respectively

[c]Mortality of animals was apparently due to fungal growth in these containers

During the second generation, production of young was significantly lower among daphnids exposed to 37.7 µg/l endosulfan than in controls and all other treatments. The previously mentioned poor survival of third generation daphnids in controls and 2.7 µg/l endosulfan is reflected also in production of young and prohibits any firm conclusions about third generation reproductive effects.

Based on the observed effects of endosulfan on survival of Daphnia magna continuously exposed through two generations, the estimated MATC for this species is >2.7 <7.0 µg/l.

Daphnia magna to trifluralin

Significant differences due to treatment were indicated from statistical analysis of data on the survival of Daphnia continuously exposed to trifluralin for 64 days. Continuous exposure to 52.7, 25.6, 14.0, and 7.2 µg/l trifluralin significantly reduced survival of daphnids with survival being cumulatively less from generation to generation (Table 17).

Production of young per Daphnia during the first two generations was significantly reduced during exposure to 52.7, 25.6 and 14.0 µg/l of trifluralin on days 22 and 38 (Table 18). On day 15 of generation one, production of young

TABLE 17. MEAN[a] PERCENT SURVIVAL OF Daphnia magna CONTINUOUSLY EXPOSED TO TRIFLURALIN FOR 64 DAYS

Mean Measured Conc. (µg/l)	GENERATION[b] I			GENERATION II			GENERATION III		
	DAY			DAY			DAY		
	8	15	22	29	38	43	50	57	64
52.7	90	70	38	35	0	0	0	0	0
25.6	90	90	48	20	5	2	0	0	0
14.0	88	83	65	85	43	13	38	35	15
7.2	90	45	43	50	23	20	8	8	0
2.4	98	93	90	90	88	50	93	93	75
Control	100	85	85	95	98	80	80[c]	80	75

[a]Each value represents the mean of four replicates

[b]Duration of exposure for generations I, II, and III were days 1-22, 22-43, 43-64, respectively

[c]On day 47, two control containers over flowed with loss of animals. Mean survival is for remaining two replicates

TABLE 18. MEAN[a] PRODUCTION OF YOUNG PER FEMALE Daphnia magna CONTINUOUSLY EXPOSED TO TRIFLURALIN FOR 64 DAYS

Mean Measured Conc. (µg/l)	GENERATION[b] I		GENERATION II		GENERATION III	
	DAY		DAY		DAY	
	15	22	38	43	57	64
52.7	3	5	0	0	0	0
25.6	15	12	8	0	0	0
14.0	11	15	18	9	16	2
7.2	11	25	32	15	16	0
2.4	18	30	31	8	23	5
Control	13	29	30	13	12	9

[a]Each value represents the mean of four replicates

[b]Duration of exposure for generations I, II, and III were days 1-22, 22-43, 43-64, respectively

was reduced only for Daphnia in 52.7 µg/l indicating possible cumulative effects on reproduction. On days 43, 57 and 64, no further differences could be attributed to toxicant concentration.

Based on the observed effects of trifluralin on survival of Daphnia magna continuously exposed through three generations, the estimated MATC for this species is >2.4 <7.2 µg/l.

Pimephales promelas to acrolein

Survival and growth of fathead minnows exposed to acrolein for 30 and 60 days were similar for all treatments (Table 19). With the exception of the A replicate of 20.8 µg/l where fish were lost due to a diluter malfunction, no significant differences in survival and growth were observed after 35 weeks exposure to acrolein.

Spawning of fathead minnows occurred in all acrolein treatments and the controls between test days 130 and 212. Spawning activity was generally below what has been observed in other chronic exposures (Table 20). With the exception of the B replicate of 20.8 µg/l (where no spawning was observed) no significant differences were observed in number of spawnings per female, number of eggs spawned per female, number of eggs per spawn, and the percent hatchability of eggs which were spawned. The complete lack of spawning activity in 20.8 µg/l acrolein may be related to the diluter malfunction which resulted in the death of fish in the A replicate of this concentration. The malfunction resulted in the emptying of the entire mixing chamber volume through the chemical cell corresponding to this concentration. It is not known how many times this occurred during an overnight period but the measured concentration during that week's sampling interval was 60 µg/l or approximately 20 µg/l higher than the mean measured concentration for the highest treatment level. We believe that the concentration of acrolein rose high enough during this short period to kill the fish in A replicate and severely stressed the fish in the B replicate resulting in their failure to spawn. The fact that fathead minnows in the highest treatment level (41.7 µg/l) survived and spawned as well as controls points to this malfunction in the dilution system as the cause for the poor performance among fish in the second highest treatment.

No second generation (F_1) fathead minnows were exposed in the 20.8 µg/l of acrolein treatment. Two larval groups exposed to 41.7 µg/l of acrolein for 60 days experienced nearly complete mortality (2% survival), and survival was significantly less than

TABLE 19. SURVIVAL AND GROWTH OF FATHEAD MINNOWS (Pimephales promelas) AFTER 30 AND 60 DAYS, AND 35 WEEKS CONTINUOUS EXPOSURE TO ACROLEIN

	Mean measured acrolein concentration (µg/l)											
	41.7 ppb		20.8		11.4		6.4		4.6		Control	
Item	A	B	A	B	A	B	A	B	A	B	A	B
30 DAYS												
% Survival[a]	93	100	93	100	100	97	95	100	100	100	100	100
Total Length (mm)	24	25	24	24	25	23	24	25	24	25	25	26
S.D.	4.0	3.8	4.4	4.0	4.6	4.5	4.9	4.9	5.2	4.1	3.5	4.2
60 DAYS												
% Survival	93	100	93	100	97	97	95	100	100	97	100	100
Total Length (mm)	33	33	31	31	32	31	32	32	32	32	31	32
S.D.	4.8	4.2	5.1	4.5	5.8	5.0	5.1	5.8	6.1	4.8	4.6	4.5
35 WEEKS												
% Survival[b]	93	100	0[c]	67	100	93	67	87	93	93	93	100
♂/♀	3/10	7/8	-	3/4	6/6	6/8	4/5	7/6	6/7	6/8	4/9	3/12
# ♂ removed	1	0	0	3	3	0	1	0	1	0	1	0
Total Length (mm)												
♂	69	61	-	67	64	66	63	61	61	61	61	59
♀	59	52	-	54	55	54	51	50	54	52	53	51
Total weight (g)												
♂	3.6	2.5	-	3.1	2.9	3.0	2.3	2.5	2.3	2.5	2.4	2.3
♀	2.0	1.2	-	1.4	1.5	1.6	1.2	1.3	1.4	1.4	1.4	1.3

[a] Survival based on 40 fish per duplicate

[b] Survival based on 15 fish per duplicate after thinning at 60 days

[c] All fish died on day 170 (diluter malfunction)

TABLE 20. SEXUAL DEVELOPMENT, SPAWNING, HATCHABILITY OF EGGS, AND SURVIVAL AND GROWTH OF OFFSPRING AFTER 30 AND 60 DAYS, FOR FATHEAD MINNOWS (Pimephales promelas) CONTINUOUSLY EXPOSED TO ACROLEIN

	Mean measured acrolein concentration (µg/l)											
	41.7 ppb		20.8		11.4		6.4		4.6		Control	
Item	A	B	A	B	A	B	A	B	A	B	A	B
# ♀	10	8	0	4	6	8	5	6	7	8	9	12
spawns/♀	1	1	-	0	1	2	5	1	2	2	1	1
eggs/♀	106	86	-	0	147	222	503	108	150	241	140	74
eggs/spawn	118	99	-	-	110	136	101	93	81	107	105	88
% Hatchability [a]	83	72	-	-	90	72	90	84	83	85	91	92
(N)	(4)	(1)	0	0	(6)	(6)	(10)	(2)	(6)	(9)	(5)	(5)
30 DAYS												
% Survival	2	-	-	-	37	29	62	35	61	44	59	45
Total Length (mm)	23	-	-	-	17	19	15	14	18	16	18	15
S.D. [b]	-	-	-	-	2.3	2.8	3.7	2.5	3.9	3.7	3.2	2.9
60 DAYS												
% Survival	2	-	-	-	37	27	43	35	50	44	49	40
Total Length (mm)	37	-	-	-	29	24	24	24	26	23	24	24
S.D.	-	-	-	-	4.2	3.3	5.9	3.8	5.3	5.0	4.5	5.8
# larval groups [c]	2	0	0	0	2	2	3	1	2	2	2	2

[a] Hatchability samples contained 50 one day old embryos

[b] Standard deviation

[c] Each larval group contained 40 one day old larvae

that of controls and lower concentration treatments.

Based on the survival of newly hatched fry during the continuous exposure of fathead minnows to various concentrations of acrolein, the estimated maximum acceptable toxicant concentration of acrolein for this species is >11.4 <41.7 µg/l.

Pimephales promelas to heptachlor

Continuous exposure for 30 days to concentrations of heptachlor as high as 1.84 µg/l had no significant effect on survival and growth of fathead minnows (Table 21). After 60 days exposure, survival was significantly reduced for fathead minnows exposed to 1.84 µg/l of heptachlor; no fish survived the 30-60 day exposure interval. After 40 weeks of exposure no significant differences were observed in survival and growth of fathead minnows exposed to the other concentrations of heptachlor and controls.

Spawning of fathead minnows occurred in all heptachlor treatments and controls between test days 141 and 261. The number of spawns per female and number of eggs per female, were similar for all tanks with surviving fish with the exception of the A replicate of 0.86 µg/l (Table 22). In this tank the number of spawns per female and eggs per female was significantly increased above all others. Parental fish in this tank experienced only 40% survival after 40 weeks and it is interesting that the few remaining fish exhibited a high level spawning activity. Possibly the reduced competition for spawning territory caused by reduced number of surviving fish was responsible for this phenomenon. No significant differences existed in hatchability of eggs from fish exposed to concentrations of heptachlor as high as 0.86 µg/l.

Statistical analysis indicated no significant differences in percent survival or growth of F_1 fathead minnows after 30 and 60 days of continuous exposure to heptachlor concentrations as high as 0.86 µg/l.

Based on these data the estimated maximum acceptable toxicant concentration of heptachlor for this species is >0.86<1.84 µg/l.

Pimephales promelas to endosulfan

Continuous exposure for 60 days to concentrations of endosulfan as high as 0.40 µg/l had no significant effect on the survival and growth of fathead minnows (Table 23). However, during the period between test days 117-145 all fish expired in both duplicate tanks receiving a mean measured concentration of 0.40 µg/l of endosulfan. At this time fish were one month from the onset of spawning and were undergoing rapid development

TABLE 21. SURVIVAL AND GROWTH OF FATHEAD MINNOWS (Pimephales promelas) AFTER 30 DAYS, 60 DAYS AND 40 WEEKS CONTINUOUS EXPOSURE TO HEPTACHLOR

Item	Mean measured heptachlor concentration (µg/l)											
	1.84		0.86		0.43		0.20		0.11		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
30 DAYS												
% Survival[a]	80	85	93	100	87	72	85	83	73	87	83	90
Total Length (mm)	17	15	17	16	16	19	17	16	19	17	18	18
S.D.[b]	2.5	2.4	1.8	2.7	2.4	2.5	2.2	1.9	2.6	2.6	2.1	2.9
60 DAYS												
% Survival	0	0	47	95	87	72	85	80	73	87	83	87
Total Length (mm)	-	-	29	28	29	33	28	29	29	28	29	28
S.D.	-	-	4.2	4.3	4.6	4.3	4.0	4.5	4.4	4.4	3.3	4.2
40 WEEKS												
% Survival[c]	0	0	40	100	93	87	93	100	87	60	73	100
♂/♀	-	-	2/4	6/9	6/8	6/7	6/8	8/7	4/9	2/7	6/5	4/11
Total Length (mm)												
♂	-	-	64	60	61	65	65	57	62	65	59	59
♀	-	-	59	54	57	58	54	52	53	55	57	55
Total weight (g)												
♂	-	-	3 1	2.3	2.6	3 3	3.0	1.9	2.5	2.7	2.1	2,4
♀	-	-	1.9	1.4	1.7	1.8	1.4	1.2	1.4	1.4	1.8	1.3

[a] Survival based on 40 fish per duplicate

[b] Standard deviation

[c] Survival based on 15 fish per duplicate after thinning at 60 days

TABLE 22. SEXUAL DEVELOPMENT, SPAWNING, HATCHABILITY OF EGGS, AND SURVIVAL AND GROWTH OF OFFSPRING AFTER 30 AND 60 DAYS, FOR FATHEAD MINNOWS (Pimephales promelas) CONTINUOUSLY EXPOSED TO HEPTACHLOR

	Mean measured heptachlor concentration (ug/l)											
	1.84		0.86		0.43		0.20		0.11		Control	
Item	A	B	A	B	A	B	A	B	A	B	A	B
# ♀	0	0	4	9	8	7	8	7	9	7	5	11
spawns/♀	-	-	21	5	5	6	5	4	4	2	5	6
eggs/♀	-	-	3582	659	691	781	580	830	559	162	495	898
eggs/spawn	-	-	173	129	145	124	122	215	140	95	108	154
% Hatchability[a]	-	-	90	89	79	86	87	88	87	85	88	88
# samples hatch	0	0	26	21	17	14	10	8	7	6	12	25
30 DAYS												
% Survival	-	-	77	50	77	90	70	54	74	78	65	68
Total Length (mm)	-	-	16	17	19	17	16	16	15	16	17	16
S.D.[b]	-	-	2.5	3.3	2.6	2.7	2.4	3.3	3.2	1.9	3.3	2.6
60 DAYS												
% Survival	-	-	77	50	73	87	61	50	68	78	64	68
Total Length (mm)	-	-	25	29	28	26	24	24	25	24	22	25
S.D.	-	-	4.3	5.8	4.5	5.6	4.0	5.0	4.5	3.0	4.6	3.7
# fry groups[c]	0	0	4	4	4	4	4	4	4	4	4	4

[a] Hatchability samples contained 50 one day old embryos

[b] Standard deviation

[c] Each larval group contained 40 one day old larvae

TABLE 23. SURVIVAL AND GROWTH OF FATHEAD MINNOWS (Pimephales promelas) AFTER 30 DAYS, 60 DAYS AND 40 WEEKS CONTINUOUS EXPOSURE TO ENDOSULFAN

Item	Mean measured endosulfan concentration (µg/l)											
	0.40		0.20		0.10		0.06		0.04		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
30 DAYS												
% Survival [a]	90	83	88	85	90	70	95	78	100	98	65	83
Mean Total Length (mm)	20	19	22	22	22	19	22	20	21	21	19	20
S.D. [b]	3.8	3.0	4.8	3.6	4.5	3.5	5.2	3.9	4.1	4.0	5.6	4.4
60 DAYS												
% Survival	88	80	88	83	90	70	93	78	98	98	60	80
Mean Total Length (mm)	27	26	29	28	28	26	29	27	26	25	28	26
S.D.	5.5	3.4	6.8	5.1	6.1	5.2	5.4	5.3	5.1	3.8	7.9	5.6
40 WEEKS												
% Survival [c]	0 [d]	0	73	40	93	73	80	80	47	33	66	80
♂/♀	-	-	4/7	2/4	5/9	6/5	5/7	4/8	2/5	2/3	7/3	6/6
Total length (mm)												
♂	-	-	63	59	58	67	59	64	59	79	65	63
♀	-	-	57	59	55	55	54	53	57	69	60	64
Total weight (g)												
♂	-	-	2.3	2.1	2.0	3.0	2.1	2.5	2.2	5.3	3.3	2.4
♀	-	-	1.5	1.7	1.3	1.2	1.2	1.3	1.5	2.3	2.2	2.1

[a] Survival based on 40 fish per duplicate

[b] Standard deviation

[c] Survival based on 15 fish per duplicate after thinning at 60 days

[d] All fish died on day 170 (diluter malfunction)

of secondary sex characteristics. Analysis of variance of percent survival after 40 weeks for fish in all remaining treatments indicated no significant differences due to treatment. We observed an infestation of fish by external parasites in the B replicate of the 0.04 µg/l treatment which we conclude contributed to the decreased survival observed in this experimental unit. Analysis of variance of total lengths and total weights of fatheads after 40 weeks exposure also revealed no significant differences due to treatment.

Spawning of fathead minnows began in most tanks with surviving adults between test days 142-162. The only exception was duplicate B of 0.04 µg/l in which no spawning occurred. Presumably, this lack of spawning was associated with the effects of the disease observed. Mean number of spawns and eggs per female fathead minnow in each duplicate tank were highly variable and precluded sensitive statistical analysis (Table 24). It is worth noting that spawning activity was increased for fathead minnows in the highest concentration of heptachlor with surviving adults (previously discussed chronic exposure) as it was in the chronic for fatheads exposed to 0.20 µg/l of endosulfan (also the highest exposure level with surviving adults). Analysis of variance revealed no significant differences due to treatment in the number of eggs per spawn or in egg hatchability for tanks where spawning had occurred. Three separate groups of eggs from control spawns were incubated in 0.40 µg/l endosulfan and only 1 percent of these eggs hatched successfully.

Analysis of variance of percent survival and mean total lengths of F_1 fathead minnows after 30 and 60 days of exposure indicated no significant differences due to exposure to 0.20 µg/l and less.

Based on these data derived from continuous exposure of fathead minnows to various concentrations of endosulfan, the maximum acceptable toxicant concentration for this species is estimated to be $>0.20<0.40$ µg/l.

Pimephales promelas to trifluralin

Analysis of variance of percent survival and total lengths of fathead minnows after 30 days exposure to trifluralin indicated no significant differences due to treatment (Table 25). After 60 days all fish continuously exposed to a mean measured concentration of 16.5 µg/l trifluralin had died. No significant differences in percent survival or total length at 60 days existed between any of the other treatments and controls. During the period between test days 125-158 all fish expired in duplicate tanks receiving a mean measured concentration of 8.2 µg/l of

TABLE 24. SEXUAL DEVELOPMENT, SPAWNING, HATCHABILITY OF EGGS, AND SURVIVAL AND GROWTH OF OFFSPRING AFTER 30 AND 60 DAYS, FOR FATHEAD MINNOWS (Pimephales promelas) CONTINUOUSLY EXPOSED TO ENDOSULFAN

	Mean measured endosulfan concentration (µg/l)											
	0.40		0.20		0.10		0.06		0.04		Control	
Item	A	B	A	B	A	B	A	B	A	B	A	B
# ♀	0	0	7	4	9	5	7	8	5	0	7	6
Mean spawns/♀	-	-	10	19	7	5	8	9	2	0	4	7
Mean eggs/♀	-	-	968	1538	559	278	709	575	104	0	490	543
Mean eggs/spawn	-	-	93	80	76	60	89	67	58	-	126	79
% Hatchability[a]	1[b]	-	74	82	87	83	84	94	86	-	82	84
# samples hatch	3	0	23	14	17	6	24	13	4	0	21	17
30 DAYS												
% Survival	-	-	70	79	83	71	80	68	74	-	68	87
Mean Total Length (mm)	-	-	18	15	16	18	18	16	17	-	18	18
S.D.[c]	-	-	4.2	2.9	2.7	3.9	2.6	3.3	3.9	-	3.1	3.7
60 DAYS												
% Survival	-	-	66	64	81	61	60	68	68	-	64	82
Mean Total Length (mm)	-	-	25	25	23	27	25	25	23	-	25	24
S.D.	-	-	5.3	5.2	4.1	5.4	2.9	4.8	4.1	-	3.8	5.5
# fry groups[d]	0	0	3	4	4	4	4	4	2	0	4	5

[a] Hatchability samples contained 50 one day old embryos

[b] Control eggs transferred to this experimental unit

[c] Standard deviation

[d] Each larval group contained 40 one day old larvae

TABLE 25. SURVIVAL AND GROWTH OF FATHEAD MINNOWS (Pimephales promelas) AFTER 30 DAYS, 60 DAYS AND 61 WEEKS CONTINUOUS EXPOSURE TO TRIFLURALIN

Item	Mean measured trifluralin concentration (µg/l)											
	16.5		8.2		5.1		1.9		1.5		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
30 DAYS												
% Survival	23	87	90	93	93	100	100	97	100	97	100	100
Total Length (mm)	19	20	24	22	20	21	22	20	17	18	21	21
S.D.[a]	6.2	5.0	7.3	5.0	5.1	5.4	5.2	4.3	4.2	3.8	4.3	3.5
60 DAYS												
% Survival[b]	0	0	85	93	93	100	100	95	100	93	100	100
Total Length (mm)	-	-	26	26	24	25	26	26	22	22	23	26
S.D.	-	-	6.2	5.2	6.4	5.8	5.2	5.2	4.7	4.3	5.1	5.0
61 WEEKS												
% Survival[c]	-	-	0	0	13	47	80	67	73	73	67	100
♂/♀	-	-	-	-	1/1	3/4	5/7	5/5	6/4	3/6	3/3	5/7
Extra ♂ removed	-	-	-	-	0	0	0	0	1	2	4	3
Total Length (mm)												
♂	-	-	-	-	60	60	66	55	65	63	60	63
♀	-	-	-	-	53	58	56	57	49	53	61	58
Total Weight (g)												
♂	-	-	-	-	2.0	3.0	3.6	2.2	3.3	3.3	3.7	2.8
♀	-	-	-	-	1.6	1.5	1.7	1.6	1.5	1.8	2.0	1.9

[a] Standard deviation

[b] Survival based on 40 fish per duplicate

[c] Survival based on 15 fish per duplicate after thinning at 60 days

trifluralin. At this time the fish were approximately one month away from the start of the spawning period and were undergoing development of secondary sex characteristics. During the period between test days 163-263 more than half of the fish died in tanks receiving 5.1 µg/l trifluralin. Surviving adults exposed to 5.1 µg/l trifluralin did not begin spawning until after day 263, approximately 100 days later than fish in controls and those exposed to 1.9 µg/l. Due to this delay, and the generally low level of spawning activity observed during the chronic exposure, the maximum photoperiod was extended approximately two months in an attempt to induce further spawning. Survival of adult fish at termination (61 weeks) was significantly reduced among fish exposed to a concentration of 5.1 µg/l of trifluralin. Analysis of variance indicated no significant differences due to treatment in total length or total weight of fathead minnows after 61 weeks exposure.

Little spawning activity occurred in any tanks with surviving adult fathead minnows in spite of the extended maximum photoperiod. What little spawning occurred was very erratic and precluded valid statistical analysis (Table 26). Percent hatchability of the few egg groups incubated was similar in all remaining concentrations of trifluralin and indicated that there were no significant differences due to treatment. Percent survival and total lengths of F_1 fathead minnows in the few places where growth chambers were started indicated that continuous exposure to 5.1 µg/l trifluralin had no effect on these parameters when compared with controls. This would be anticipated considering that F_0 fathead minnows exposed to 5.1 µg/l did not experience reduction in survival until after 163 days of exposure and this period of mortality probably corresponded with secondary sexual development.

Based on these data derived from continuous exposure of fathead minnows to various concentrations of trifluralin, the maximum acceptable toxicant concentration for this species is >1.95<5.1 µg/l.

RESIDUE ANALYSIS

Three samples of pooled eviscerated carcasses of terminated adult fathead minnows in each concentration of trifluralin and heptachlor with surviving adults were analyzed to determine residues in the fish. Results of these analyses (Table 27) indicate that the amount of trifluralin residue accumulated is approximately 1000X the concentration in water, is directly proportional to the level of exposure, and that this response is essentially linear over the range of concentrations tested.

TABLE 26. SPAWNING RESULTS, EGG HATCHABILITY, AND SURVIVAL AND GROWTH OF FATHEAD MINNOW (Pimephales promelas) FRY AFTER 30 AND 60 DAYS EXPOSURE TO VARIOUS CONCENTRATIONS OF TRIFLURALIN

Item	Mean measured trifluralin concentration (µg/l)											
	16.5		8.2		5.1		1.9		1.5		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
# ♀ spawning	0	0	0	0	5	4	8	6	5	6	3	7
Mean spawning/♀	0	0	0	0	3	7	2	2	0	1	2	3
Mean eggs spawned/♀	-	-	-	-	225	432	64	104	0	20	55	229
Mean eggs/spawn	-	-	-	-	63	60	36	42	-	20	23	76
% Hatchability[a]	-	-	-	-	89	73	95	75	-	-	94	88
# Hatchability samples	0	0	0	0								
30 DAYS												
% Survival					83	60	56	-	-	-	-	84
Mean Total Length (mm)					16	19	19	-	-	-	-	17
S.D.[b]					3.5	3.2	2.1	-	-			3.6
60 DAYS												
% Survival					62	59	56	-	-	-	-	74
Mean Total Length (mm)					21	28	28	-	-	-	-	22
S.D.					5.4	4.8	4.6	-				5.7
# fry groups[c]					2	2	3	0	0	0	0	2

[a] Hatchability samples contained 50 one day old embryos

[b] Standard deviation

[c] Each larval group contained 40 one day old larvae

TABLE 27. MEAN MEASURED CONCENTRATIONS OF HEPTACHLOR (+ EPOXIDE) AND TRIFLURALIN IN WATER (µg/l) AND IN THE EVISCERATED CARCASS (µg/kg) OF FATHEAD MINNOWS (Pimephales promelas) CONTINUOUSLY EXPOSED TO THE PESTICIDES IN SEPARATE SYSTEMS

Item	Tank Number 1	2	3	4	5	Control
HEPTACHLOR						
(276 days) water	1.84	0.86	0.43	0.25	0.11	-
Carcass	*	17,733 ± 6752	10,240 ± 6841	4 250 ± 577	2,450 ± 1131	250 ± 62
Bioconcentration factor (X)	*	20,620	23,814	17,000	22,273	-
TRIFLURALIN						
(425 days) water	16.5	8.2	5.1	1.9	1.5	
Carcass	*	*	4,900 ± 3500	2,533 ± 1401	1,333 ± 351	293 ± 94
Bioconcentration factor (X)	*	*	961	1,333	889	-

* All fish died during exposure

Heptachlor residues in fish are presented as the combined residues of heptachlor and heptachlor epoxide. Heptachlor epoxide residues in fish generally constituted 10-24 percent of the total residue. The amount of heptachlor residue accumulated is approximately 20,000X the concentration in the water and the residue appears proportional to the level of exposure with the response apparently linear over the range of concentrations tested.

CALCULATION OF APPLICATION FACTORS

Estimated LC50 values, maximum acceptable toxicant concentrations, and the application factors derived therefrom are summarized for the four pesticides and the two species undergoing chronic exposures (Table 28, 29). Application factors determining the relationship between the acute and chronic toxicity of each compound for Daphnia magna, are calculated using the MATC and the 48-hour LC50. Similar application factors are calculated for fathead minnows utilizing the MATC and the incipient LC50 which Eaton (1970) suggests to be a better measure of acute toxicity for this type of calculation.

In the acrolein, heptachlor, and trifluralin exposures application factors for Daphnia were similar to those calculated for fathead minnows. However, the application factor calculated for Daphnia exposed to endosulfan is an order of magnitude lower than that calculated for fathead minnows and endosulfan.

TABLE 28. ACUTE AND CHRONIC TOXICITY VALUES FOR THE WATER FLEA (Daphnia magna) AND CALCULATED APPLICATION FACTORS FOR EACH PESTICIDE

Pesticide	48-hour LC50 (µg/l)	MATC (µg/l)	Limits on Application factors (µg/l)
Acrolein	57 (20-99)	>16.9<33.6	0.30-0.59
Heptachlor	78 (46-113)	>12.5<25.0	0.16-0.32
Endosulfan	166 (126-219)	>2.7<7.0	0.016-0.042
Trifluralin	193 (115-327)	>2.4<7.2	0.012-0.037

TABLE 29. ACUTE AND CHRONIC TOXICITY VALUES FOR THE FATHEAD MINNOW (Pimephales promelas) AND CALCULATED APPLICATION FACTORS FOR EACH PESTICIDE

Pesticide	Incipient LC50 (µg/l)	MATC (µg/l)	Limits on Application factors (µg/l)
Acrolein	84 (54-130)	>11.4<41.7	0.14-0.50
Heptachlor	7.02 (5.76-8.54)	>0.86<1.84	0.12-0.26
Endosulfan	0.86 (0.52-1.40	>0.20<0.40	0.23-0.47
Trifluralin	115 (48-211)	>1.9<5.1	0.017-0.044

SECTION VI

DISCUSSION

The incipient LC50 of acrolein to fathead minnows (84 µg/l) which we determined in a flowing bioassay is in basic agreement with the values which have been previously reported. Burdick *et al.* (1964) reported 24-hour LC50 values of 46 µg/l of acrolein for brown trout and 79 µg/l for bluegill. Alabaster (1969) reported a 24-hour TL_m of 140 µg/l of acrolein for harlequin fish. Louder and McCoy (1962) reported a narrow range of acrolein toxicity with five species of warmwater fishes including the fathead minnow with 24-hour TL_m values ranging from 62-183 µg/l. It is not surprising that our LC50 value in a 6-day flowing study agrees with 24-hour static values because the majority of mortalities observed in the 6-day test occurred within the first 48 hours of exposure. No comparable toxicity data were found for invertebrates and acrolein with which to compare our results for *Daphnia magna*.

The 48-hour LC50 of 78 µg/l of heptachlor which we determined for *Daphnia magna* is higher than the 48-hour LC50 values of 42 and 47 µg/l which Sanders and Cope (1966) reported for *Daphnia pulex* and *Simocephalus serrulatus*, respectively. It appears that temperature as well as species differences are responsible for the discrepancy since our value was determined at 19°C while Sanders and Cope determined their values at 15.5°C. These authors provide the evidence that probably temperature caused the difference by reporting a 48-hour LC50 of 80 µg/l for *Simocephalus serrulatus* exposed to heptachlor at 70°F or 21°C. This value is essentially identical to the 78 µg/l which we determined for *Daphnia magna* at 19°C, and is a two fold increase over the value which the authors reported for the same species at the lower temperature.

As might be expected the incipient LC50 of heptachlor to fathead minnow (7.0 µg/l) which we determined in a 10 day flowing bioassay is much lower than the 96-hour TL_m of 130 µg/l which Henderson *et al.* (1960) determined for the same species. The much smaller size of our fish, longer exposure period, and continuous renewel of heptachlor in the flowing system all contribute to the lower value. In our prelininary investigations a 96-hour static exposure of fathead minnows resulted in a calculated LC50 of 157 µg/l which is in good agreement with the 130 µg/l value reported by Henderson *et al.*

The 48-hour LC50 of endosulfan for Daphnia magna which we estimated at 166 µg/l, is approximately three times greater than the 62 µg/l reported by Schoettger (1970) for a similar exposure of the same species. A possible explanation for the discrepancy could be the age of the test organisms. Daphnids in our acute studies were <24 hour old and Schoettger does not specify age of Daphnia magna which he used. The incipient LC50 of endosulfan which we determined for fathead minnows (0.86 µg/l) is lower than two previously reported acute values, again to be expected due to the constant flow and longer duration of our study. Pickering and Henderson (1966) estimated the 96-hour TL_m of thiodan (registered tradename of endosulfan) to bluegills as 3.3 µg/l, while Schoettger (1970) estimated the 120-hour LC50 to white suckers to be 2.1 µg/l. Fathead minnows in both our acute and chronic exposures proved to be much more susceptable to endosulfan than were Daphnia magna. Schoettger (1970) reported a general tendency of most aquatic invertebrates to be less sensitive than fish to thiodan (endosulfan).

The 48-hour LC50 of 193 µg/l of trifluralin which we calculated for Daphnia magna is 2-3 times lower than the 560 µg/l which Sanders (1970) reported for the same species in a similar exposure to trifluralin. Our value is in better agreement with the 240 µg/l trifluralin which was reported by Sanders and Cope (1966) as the 48-hour EC50 for Daphnia pulex.

The incipient LC50 of trifluralin to fathead minnows we estimated as 115 µg/l is in fair agreement with Park and Worth (1965) who report a 96-hour LC50 of 93.4 µg/l of trifluralin with the same species. Trifluralin seems to be similar to endosulfan in that invertebrate species appear less sensitive than fish species.

The bioconcentration factors (i.e. 17,000-23,000 X) in fish continuously exposed to heptachlor are consistent with those reported for other organochlorine compounds such as DDT (Macek and Korn,1970), dieldrin (Bionomics, E G & G, Inc., unpublished data), hexachlorbenzene (Parrish et al., 1975), Aroclor 1254 (Hansen et al., 1971), toxaphene (Mayer et al., 1975) and tetrachlorobiphenyl (Branson et al., 1974). The bioconcentration factors for all of these compounds, including heptachlor, are generally 1-2 orders of magnitude higher than those determined for other types of agricultural chemicals (Macek et al., 1975).

The observed residues in the tissues of fish continuously exposed to concentrations of heptachlor which appear to be safe for fathead minnow (i.e. <1.0 µg/l) are similar to concentrations of heptachlor residues which Henderson et al.

(1969, 1971) reported in field collected samples. This observation suggests that the concentrations of heptachlor residues occurring in many natural waters which these authors sampled are probably below the MATC for fathead minnows. However, in view of the variability among the estimated limits for the MATC for lindane and aquatic organisms (Macek et al., 1975) it is conceivable that some subtle harmful effects due to chronic exposure to heptachlor residues may be occurring in contaminated surface waters. Since similar information on the occurrence of endosulfan and trifluralin residues in natural waters is not readily available, an assessment of the hazard of chronic exposure of aquatic organisms to these compounds is not possible. However, the relatively high toxicity of these compounds to aquatic species suggests the need for some monitoring information to quantify existing levels of endosulfan and trifluralin residues in surface waters.

SECTION VII

REFERENCES

Alabaster, J.S. 1969. Survival of fish in 164 herbicides, insecticides, fungicides, wetting agents and miscellaneous substances. Int. Pest. Cont. 7(1): 29-35.

American Public Health Association. 1971. Standard Method for the Examination of Water and Wastewater. 13th ed. Am. Public Health Assoc., New York. 874pp.

Andrews, A.K., C.C. Van Valin, and B. Stebbings. 1966. Some effects of heptachlor on bluegills (Lepomis macrochirus). Trans. Am. Fish. Soc. 95(3): 247-309.

Arthur, J.W. 1970. Chronic effects of linear alkylate sulfonate detergent on Gammarus pseudolinmaeus, Campeloma decisum and Physa integra. Water Research 4: 251-257.

Arthur, J.W., A.E. Lemke, V.R. Mattson, and B.J. Halligan. 1973. Toxicity of sodium nitrilotriacetate (NTA) to the fathead minnow and an amphipod in soft water. Water Research 8: 187-193.

Battelle - Northwest Laboratories. 1970. Degradation and depletion of herbicides in drainage water and accumulation of residues in crops irrigated with treated water. Unpublished report. Richland, Wash. 45pp.

Biesinger, K.E. and G.M. Christensen. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of Daphnia magna. J. Fish. Res. Bd. Canada 29: 1691-1700.

Bioassay Committee. 1971. Recommended bioassay procedures for fathead minnow (Pimephales promelas, Rafinesque) chronic tests. U.S. Environmental Protection Agency, Duluth, Minnesota 13pp.

Branson, D.R., G.E. Blan, H.C. Alexander, D.R. Thielen, and W.B. Neeley. 1974. A bioconcentration test: Steady-state concentrations of 2,2',4,4'-tetrachlorobiophenyl in trout. Trans. Am. Fish. Soc. (In Press).

Bridges, W.R. 1965. Effects of time and temperature on the toxicity of heptachlor and kepone to redear sunfish. U.S. Public Health Service, Cincinnati, Ohio. PHS-999-WP-25.

Bruce, W.N. and G.C. Decker. 1966. Insecticide residues in soybeans grown in soil containing various concentrations of aldrin, dieldrin, heptachlor, and heptachlor epoxide. J. Agr. Food Chem. 14(4): 395ff.

Burdick, G.E., H.J. Dean, and E.T. Harris. 1964. Toxicity of aqualin to fingerling brown trout and bluegills. N.Y. Fish and Game J. 11(2): 106-114.

Eaton, J.G. 1970. Chronic malathion toxicity of the bluegill (Lepomis macrochirus, Rafinesque). Water Research 4: 673-684.

Eichelberger, J.W. and J.J. Lichtenberg. 1971. Persistence of pesticides in river water. Envir. Sci. Tech. 5: 541-544.

Eisler, R. 1970. Acute toxicity of organochlorine and organophosphorus insecticides to estuarine fishes. U.S. Dept. Interior Bur. Sport Fish. Wildlife, Technical Report 46, 11pp.

Frear, D.E.H. and J.E. Boyd. 1967. Use of Daphnia magna for the microbioassay of pesticides I. Development of standardized techniques for rearing Daphnia and preparation of dosage mortality curves for pesticides. J. Econ. Ent. 60(5): 1228-1236.

Greve, P.A. and S.L. Wit. 1971. Endosulfan in the Rhine River. J. Water Poll. Cont. Fed. 43(12): 2338-2348.

Hannon, M.R., Y.A. Greichur, and R.L. Applegate. 1970. Ecological distribution of pesticides in Lake Poinsett, South Dakota. Trans. Am. Fish. Soc. 99(3): 496-500.

Hansen, D.L., P.R. Parrish, L.I. Lowe, A.J. Wilson Jr., and P.D. Wilson. 1971. Chronic toxicity uptake and retention of Aroclor 1254 in two estuarine fishes. Bull. Env. Contam. Toxicol. 6(2): 113-119.

Harris, C.R. and W.W. Sans. 1972. Behavior of heptachlor epoxide in soil. J. Econ. Ent. 65(4): 336ff.

Henderson, C., H.Q. Pickering, and C.M. Tarzwell. 1959. Relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. Trans. Am. Fish. Soc. 88(1): 23-32.

_____. 1960. The toxicity of organic phosphorus and chlorinated hydrocarbon insecticides in fish. U.S. Public Service, Washington, D.C. Technical Report W60-3.

Henderson, C., W.L. Johnson, and A. Inglis. 1969. Organochlorine insecticide residues in fish. Pest. Mont. J. 3(3): 145-171.

_____. 1971. Residues in fish, wildlife and estuaries. Pest. Mont. J. 5(1): 1-11.

Hesselberg, R.J. and J.L. Johnson. 1972. Column extraction of pesticide from fish food and mud. Bull. Env. Contam. Toxicol. 7(2/3): 115-120.

King, R.L., N.A. Clark, and R.W. Hemken. 1966. Distribution movement, and persistence of heptachlor and its epoxide in alfalfa plants and soil. J. Agr. Food Chem. 14(1): 62ff.

Kruppa, R.F., R.S. Henly, and D.L. Smead. 1967. Improved gas chromatography packings with fluidized drying. Applied Science Laboratories, Inc. Tech. Bull. No. 2A.

Lawrence, J.M. 1966. Effects of soil applied trifluralin in fish production in pools. Proc. Ann. Meeting Weed Soc. Amer., St. Louis, Missouri.

Litchtenberg, J.J., J.W. Eichelberger, R.C. Dressman, and J.E. Longbottom. 1969. Pesticides in surface water of the United Sates, a five-year survey. Pest. Mont. J. 3: 28ff.

Litchtenstein, E.P. and K.K. Schulz. 1965. Residues of aldrin and heptachlor in soils and their translocation into various crops. J. Agr. Food Chem 13(1): 57ff.

Litchtenstein, E.P., K.K. Schulz, T.W. Fuhremann, and T.T. Leang. 1970. Degradation of aldrin and heptachlor in field soils during a ten year period. J. Agr. Food Chem. 18(1): 100-106.

Louder, D.E. and E.G. McCoy. 1962. Preliminary investigations in the use of aqualin for collecting fishes. Proc. 16th Ann. Conf. Southeast. Assoc. Game and Fish Comm., Charleston, South Carolina, pp. 240-242.

Macek, K.J., M.E. Barrows, R.F. Krasny, and B.H. Sleight. 1975. Biocentration of 14C-pesticides by bluegill sunfish during continuous aqueous exposure. Proc. Symposium on Structure Activity Correlation in Studies of Toxicity and Bioconcentration with Aquatic Organisms, Canada Centre for Inland Waters, Burlington, Ont. (March).

Macek, K.J., K.S. Buxton, S.K. Derr, J.W. Dean, and S. Sauter. 1976a. Chronic toxicity of lindane to selected aquatic invertebrates and fishes. U.S. Environmental Protection Agency, Duluth, Minnesota. Ecological Research Series (In Press).

Macek, K.J., K.S. Buxton, S. Sauter, S. Gnilka, and J.W. Dean. 1976b. Chronic toxicity of atrazine to selected aquatic invertebrages and fishes. U.S. Environmental Protection Agency, Duluth, Minnesota. Ecological Research Series (In Press).

Macek, K.J., C. Hutchinson, and O.B. Cope. 1969. The effects of of temperature on the susceptibility of bluegills and rainbow trout to selected pesticides. Bull Env. Contam. Toxicol. 4(3): 174-183.

Macek, K.J. and S. Korn. 1970. Significance of the food chain in DDT accumulation of fish. J. Fish. Res. Bd. Canada 27 (8): 1496-1498.

Mayer, F.L. P.M. Mehrle, and W.P. Dwyer. 1975. Toxaphene effects on reproduction, growth and mortality of brook trout. U.S. Environmental Protection Agency, Duluth, Minnesota. Ecological Research Series EPA-600/3-75-013. 51pp.

McKim, J.M. and D.A. Benoit. 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (Salvelinus fontinalis). J. Fish. Res. Bd. Canada 28: 655-662.

Mount, D.I. 1968. Chronic toxicity of copper to fathead minnows (Pimephales promelas, Rafinesque). Water Research 2: 215-223.

Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. Water Research 1: 21-29.

Mount, D.I. and C.E. Stephan. 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96: 185-193.

National Academy of Sciences. 1973. Water Quality Criteria 1972. A report of the Committee on Water Quality Criteria Environmental Studies Board National Academy of Engineering, Washington, D.C. 553pp.

Overbeck, T.V., 1962. Method of controlling aquatic plants. Wat. Poll. Abst. 35(12): No. 2410.

Parks, S.M. and H.M. Worth. 1965. The effects of trifluralin to fish. Proc. Southern Weed Conference, Dallis, Texas. Vol. 18, pp 469-474.

Parrish, P.R., G.H. Cook, and J.M. Patrick. 1975. Hexachlorobenzene: Effects on several estuarine animals. Proc. 28th Ann. Conf. Southeast. Assoc. Game and Fish Comm., St. Louis, Missouri (In Press).

Pickering, Q.H. and C. Henderson. 1966. The acute toxicity of some pesticides to fish. Ohio J. Sci. 66: 508-513.

Sanders, H.O. 1970. Toxicities of some herbicides to six species of freshwater crustaceans. J. Water Poll. Control Fed. 42 (8): 1544-1550.

Sanders, H.O. and O.B. Cope. 1966. Toxicities of several pesticides to two species of cladocerans. Trans. Am. Fish. Soc. 95: 165-169.

Sanders H.O. and O.B. Cope. 1968. The relative toxicity of several pesticides to naiads of three species of stoneflies. Limnol. Ocean 13(1): 112-117.

Schoettger, R.A. 1970. Toxicity of Thiodan in several fish and and aquatic invertebrates. In: Investigations in Fish Control, No. 35. U.S. Dept. of Interior, Bur. Sport Fish. and Wildlife, Washington, D.C. 31pp.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw Hill, New York. 471 pp.

U.S. Department of Interior. 1972. Handbook of Procedures for Pesticide Residue Analysis #65. U.S. Dept. of Interior, Bur. Sport Fish. and Wildlife, Washington, D.C.